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[*Authors alone are responsible for all opinions expressed in their Communications.*]

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No. 13.

THE MEASUREMENT OF THE CURRENT GENERATED BY RECTIFIER PHOTO-CELLS.

By H. H. POOLE

AND

W. R. G. ATKINS, F.R.S.

[Read November 27. Published separately November 28, 1934.]

Rectifier photo-cells yield much larger currents than vacuum cells of the emission type for the same intensity of illumination. Moreover, the absence of high potentials removes many insulation difficulties which may arise when measurements of weak daylight have to be made in the field, or at sea, in adverse weather conditions. The usual methods of measuring these currents, however, suffer from the limitation that, if high sensitivity is required, the measuring instrument employed must possess a fairly high resistance. This is of no consequence in the case of emission cells, as the potential drop across this resistance is negligible compared with the potential supplied by the battery, which must be sufficient to ensure approximate saturation, and in our work has generally been about 60 volts. With rectifier cells, however, no external source of potential is used, the cell acting approximately as a self-acting source of current, increasing with the illumination, shunted by an internal leakage resistance which decreases with increase of illumination, or with rise in temperature. It is obvious, then, that the use of a high-resistance measuring instrument increases the importance of this leakage current, and introduces two objectionable features:—(*a*) The light/current characteristic deviates much more from linearity, the external current tending towards a limit in very bright light; and (*b*) temperature errors are much more important.

For this reason we have always used a 10-ohm galvanometer with suitable shunts for shore work, with a Bergmann (Weston) cell, only using higher resistance instruments (up to 320 ohms) for measuring low illuminations in shaded sites, where the curvature of the characteristic becomes negligible (3). For submarine work we have used our potentiometer amplifier telephone method to measure the drop in potential across a resistance in series with the cell (4). This resistance was 10 ohms for bright light, but needed to be increased to 100, 1,000, or 10,000 ohms for measurements under colour filters in deep water. The characteristic must be obtained for the cell for each resistance, and the

corresponding correction applied to the readings. It is preferable always to use the lowest resistance that will give the requisite sensitivity, but there is a great temptation to increase the resistance and so facilitate quick and accurate measurements by increasing the potential drop to be measured.

Campbell and Freeth (1) have recently described a method of using rectifier cells which has the effect of using a measuring instrument of zero resistance, and thus greatly reducing the effects of internal leakage in the cell. The effects are probably not entirely eliminated as, owing to the resistance of the conducting film forming the front electrode of the cell, parts of it must still differ somewhat in potential from the back plate, even though the terminals are at the same potential.

Their circuit is shown in Fig. 1. Here the source of variable potential *P* is adjusted to give a current, measured by the microammeter or galvanometer, *M*, exactly equal to that provided by the cell, the balance being shown by the galvanometer *G*, which is used solely as a null-point indicator. It will be observed that the resistances of *M* and *G* do not affect the cell, whose terminals are brought to the same potential when the circuit is balanced. Campbell and Freeth have shown that temperature errors are greatly reduced, if not entirely eliminated, by this simple and elegant method. Moreover, the curvature of the light/current characteristic corresponds to that for a galvanometer of zero resistance.

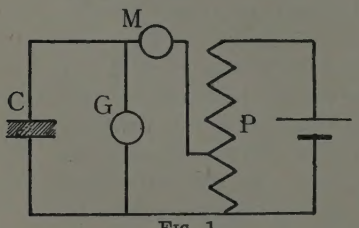

FIG. 1.

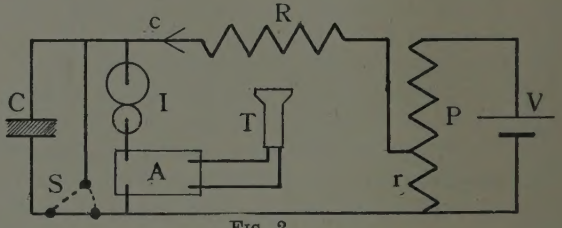

FIG. 2.

The need for the use of two galvanometers, a potential divider, and a battery, detracts somewhat from the portability of the arrangement, which may be a serious disadvantage for measurements in woods, etc. It occurred to us, however, that with the simplest modification our potentiometer amplifier apparatus could be altered to obtain all the advantages of this method for use at sea (5). We are here debarred by the motion of the ship from using a galvanometer to measure the current, so we replace *M* by a known resistance, *R*, which may conveniently be varied from 100 to 100,000 ohms according to the illumination to be measured, and find the current from the reading of the potentiometer used as the source of potential. For sea work *G* is replaced by our interrupter amplifier telephone combination, which can readily be made sensitive to 10^{-9} ampere or less. For work in the laboratory a sensitive galvanometer combined, if required, with an electrometer valve amplifier may be used, so that great sensitivity and accuracy of current measurement may be obtained.

Figure 2 shows the circuit as arranged for marine work. Here C is the cell, I the interrupter, which has already been described (2), A a 2- or 3-valve amplifier, and T telephones. R is a resistance which can readily be set at 10, 100, 1,000, 10,000, or any multiple of 10,000 up to 100,000 ohms. With the latter and the Tinsley Potentiometer P in the $\times$ 0·1 position 1 division of the potentiometer slide wire corresponds to 10^{-9} ampere, which is near the limit that can be detected with our apparatus under normal working conditions. With suitable precautions to avoid interference from extraneous noises, A.C. mains, etc., and under sufficiently dry atmospheric conditions, to prevent errors from surface leaks, the sensitivity can be considerably increased, and it would be advantageous to increase R to at least a megohm.

It is convenient to connect the leads to the "galvanometer" terminals of the potentiometer and to short-circuit the "potential" terminals, which of course are in series with the latter. By this means we can use the telephone to set the potentiometer against its standard cell (not shown in the figure) by merely cutting out the rectifier cell by the two-way switch S, and moving the potentiometer selector switch (not shown) to the "standard cell" position, in which the short-circuited "potential" terminals are cut out and the standard cell inserted instead. R, which during this setting forms part of the standard cell circuit, may, if desired, be reduced to 10 ohms, though this makes little difference, and ample sensitivity is generally available. S is actually a porcelain-covered electric light two-way or "corridor" switch, as used at sea with emission cells. When in the "off" position the cell is short-circuited. Two of these switches with suitable terminals are fitted in our apparatus, so that we can instantly change to either of two cells used for comparing the daylight on deck and under water.

We must consider the possibility of errors involved in this use of the potentiometer, and the corrections necessitated by the fact that the potentiometer system itself is now out of balance.

It is evident that, since the current flowing through R is calculated from the known resistances, extraneous resistances must be reduced to negligible proportions. The most serious possibility appears to be doubtful contacts with the potentiometer slide wire, or at the selector switch. Such small additional resistances would not affect the accuracy of the usual balanced potentiometer, but might introduce appreciable errors into the calculation of the out-of-balance current, which is used in this case. Tests with a 10-ohm microammeter showed, however, that the additional resistance was a very small fraction of an ohm, and the contacts appeared to be quite reliable. Accordingly, with $R = 100$ ohms, the smallest resistance which we should use with a Weston cell for the measurement of sunlight, this error should not exceed a small fraction of 1 per cent., provided that reasonable precautions are taken to ensure that the potentiometer contacts remain in good condition.

In calculating the current, c, flowing through R and through the cell, we may evidently disregard the resistance of the latter and that of the amplifier

interrupter system, since, as there is no P.D. between the terminals of the latter, we may imagine it to be short-circuited without affecting any of the currents flowing.

Accordingly, $$c = \frac{r}{R+r} \times \frac{V}{\frac{Rr}{R+r} + P - r},$$

where V is the voltage of the potentiometer accumulator, P the total resistance of the potentiometer, and r the resistance included between the contact points on the potentiometer. This may be written

$$c = \frac{E}{R\left[1 + \frac{P}{R}\left(\frac{E}{V} - \frac{E^2}{V^2}\right)\right]} = \frac{c'}{1 + \frac{P}{R}\left(\frac{E}{V} - \frac{E^2}{V^2}\right)} = Fc',$$

where E is the potentiometer reading, c' is the apparent or uncorrected current E/R, and F is a correction factor, not very much less than unity.

For the Tinsley potentiometer on the $\times$ 1 range $R = 40$ ohms, $V = 2{\cdot}0$ volts, the small excess of voltage of the accumulator above 2·0 being absorbed in the adjusting rheostat, which, being only a fraction of an ohm, would have little effect on the value of the correction. On the $\times$ 0·1 range an extra 360 ohms is inserted in series and 400/9, *i.e.*, 44·44 . . ., ohms in parallel. If we again neglect the small change in P.D. across the balancing rheostat caused by the photo-cell current, we may neglect the effect of the shunt, since the latter carries a sensibly constant current. For this range, therefore, the effective value of P is 400 ohms.

Fig. 3 shows how the correction factor F varies with E. Curve A corresponds to $R = 100$ ohms on the $\times$ 1 range or $R = 1{,}000$ ohms on the $\times$ 0·1 range. B corresponds to 1,000 ohms, $\times$ 1, or 10,000 ohms, $\times$ 0·1. For higher resistances we may take $F = 1$. The values of E are only plotted up to 1 volt, as it is easy to see that the same value of F occurs at voltages E and $2 - E$, so that the correction for any possible voltage can be found immediately. On the $\times$ 0·1 range E is, of course, always less than 0·2 volt.

From the practical point of view where large numbers of measurements have to be made the need for applying this correction appears to be the chief objection to the method. It is, however, far out-weighed by the great advantage of being able to measure all illuminations, from the most intense sunlight down to a small fraction of a metre candle, without altering the light/current characteristic of the cell by the introduction of different series resistances.

We have not yet had an opportunity of using this method at sea, but laboratory tests with the marine telephone apparatus showed that currents could readily be measured down to 10^{-9} ampere without taking any precautions to prevent the interference due to A.C. mains. For the Weston cell under opal glass this corresponds to about $1{\cdot}5 \times 10^{-2}$ metre candle.

As a further test of the reliability of the method we have compared the

current yielded by a bare Weston cell for this circuit of zero effective resistance with that flowing through resistances of 10, 100, 1,000, and 10,000 ohms, for a long range of illuminations. In this case a galvanometer was used as a null-point indicator. The cell was mounted on the photometer bench behind a water cell, the source of light being a 150 watt 60 volt gas-filled projector lamp run off a large storage battery at 54·7 volts. The usual corrections were made for the optical effect of the water cell, and for the sizes of the cell and the source. This correction amounts to about 8 per cent. at the shortest distance (7 cm.) used, and as its magnitude depends on the angular distribution of the light from the filament, some uncertainty in the higher illuminations is inevitable.

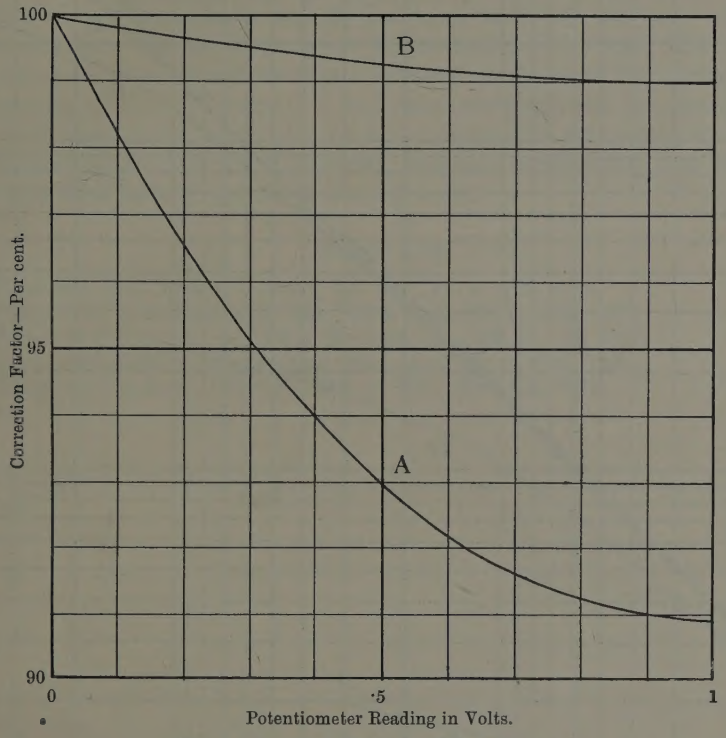

FIG. 3.—Correction Factor for finding current.

The results are plotted in Fig. 4, logarithmic scales being used owing to the length of the ranges to be covered. This is in some ways a disadvantage, as it rather masks the fall in sensitivity with illumination. The dotted line at 45° indicates the behaviour of a cell giving a current strictly proportional to the light. The other graphs *A*, *B*, *C*, *D*, and *E*, give the characteristics for the Campbell-Freeth zero resistance circuit, and series resistances of 10 ohms, 100

ohms, 1,000 ohms, and 10,000 ohms, respectively. The graphs are plotted by the simple method of joining consecutive observation points by right lines, without any attempt at smoothing. The highest illumination, 37,000 m.c., falling on the bare cell is about equal to full sunlight on the cell, as usually mounted, behind opal glass.

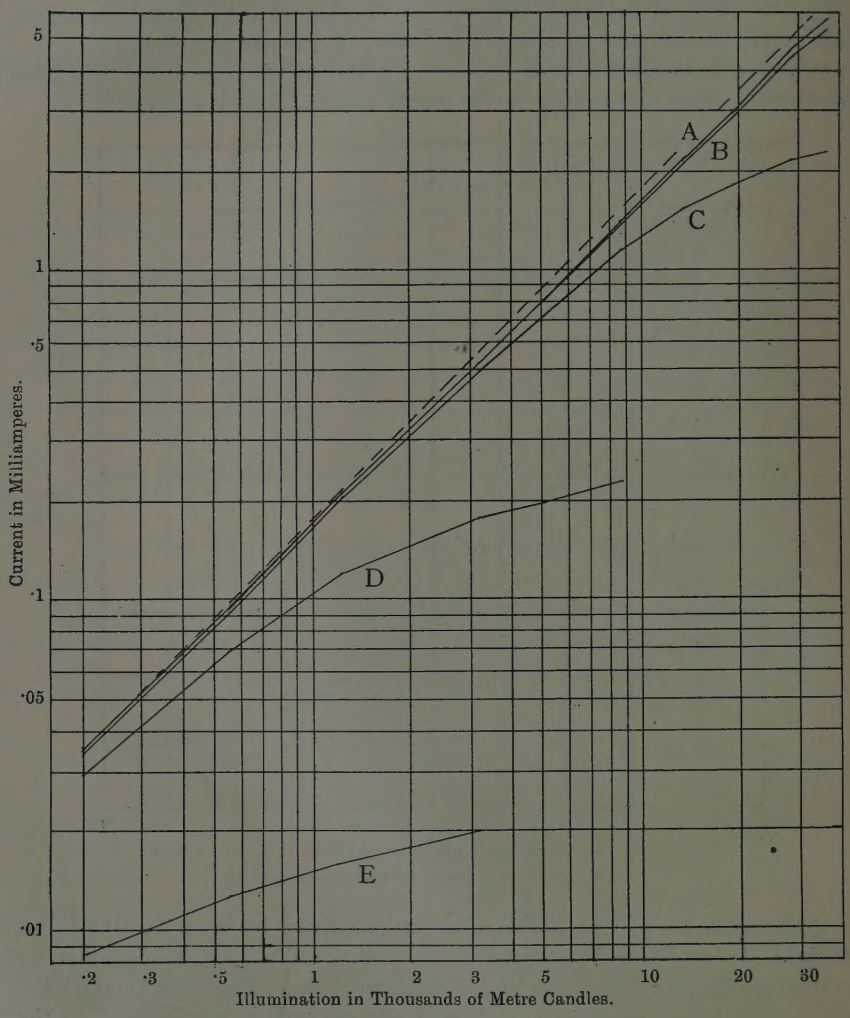

Fig. 4.—Light/Current Characteristics for Weston cell No. 21107–4 for various series resistances. A, zero (Campbell-Freeth circuit). B, 10 ohms. C, 100 ohms. D, 1,000 ohms. E, 10,000 ohms. The dotted line at 45° represents a truly linear response.

It will be seen that the zero resistance circuit approaches somewhat more closely to true proportionality than the 10-ohm circuit which we have hitherto used as a standard practice. The correction which must be made to allow for the curvature of the characteristic is, thus, smaller with the Campbell-Freeth circuit. The difference is not very great and becomes inappreciable below 5,000 m.c.

SUMMARY.

A description is given of a modified form of the circuit described by Campbell and Freeth for the measurement of the current generated by rectifier photo-cells. The modification consists in the deduction of the current from the setting of the potentiometer and the resistance in circuit, instead of its direct measurement. This enables accurate measurements to be made of a range of illuminations from a small fraction of a metre candle up to full sunlight while preserving all the advantages of a zero-resistance measuring instrument. It is especially suitable for work at sea, for which purpose the null-point galvanometer is replaced by an interrupter, amplifier, telephone combination, thus rendering the apparatus suitable for use in a small vessel.

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5. POOLE and ATKINS.—Nature, **134**, p. 810, Nov. 24, 1934.

No. 14.

THE CHEMICAL CONSTITUENTS OF LICHENS FOUND IN IRELAND.

LECANORA GANGALEOIDES—Part I.

By JOSEPHINE HARDIMAN, M.Sc.; J. KEANE, Ph.D.;

AND

T. J. NOLAN, B.A., D.Sc.,

University College, Dublin.

[Read November 27, 1934. Published separately January 4, 1935.]

The lichen *Lecanora gangaleoides* was collected on the maritime rocks in Howth, on the promontory with a north-eastern aspect. The same lichen was found in quantity in Killiney, on the western side overlooking the golf links. It consists of a greyish-white incrustation abundantly covered with blackish apothecia. It was identified for us by Dr. P. O'Connor of the Natural History Division of the National Museum.

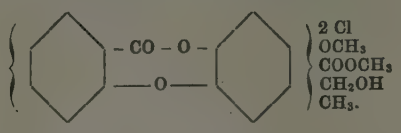
The lichen was successively extracted with ether, acetone, chloroform, and alcohol. From the ether extract two compounds were isolated; one of these consisted of white prisms m.p. 195–197° C., and appears to be a mixture of atranorin and chloratranorin. This compound was also the principal constituent of the acetone and chloroform fractions. A second product isolated from the ether extract melted at 214–215° C., and gave analyses corresponding to the formula $C_{18}H_{14}Cl_2O_7$. This compound we propose to name Gangaleoidin. There appeared to be evidence of a third higher melting constituent in the ether extract, but the quantity isolated was too small to allow of examination. From the alcohol extract no sugar alcohol was isolated, but evidence was obtained of the existence in it of a water-soluble ester or lactone.

We are at present carrying out a more complete investigation of this lichen using larger quantities of material.

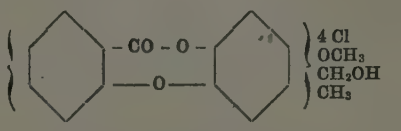
Gangaleoidin contains two methoxyl groups, and forms a monoacetyl derivative on heating with acetic anhydride and sodium acetate. The same acetate is obtained if acetic anhydride and sulphuric acid are employed, so that there does not appear to be any aldehyde group present in the substance. It is insoluble in sodium bicarbonate, and is only very slowly dissolved by 10 per cent. aqueous potash. The crystals are stained a faint yellow colour by concentrated sulphuric acid, in which they dissolve to a colourless solution. An alcoholic solution of Gangaleoidin gives no colouration with ferric chloride, and the crystals on treatment with a solution of bleaching powder show no reaction.

On treatment with methyl alcoholic potash in the cold, and subsequent acidification, a compound is obtained which is insoluble in sodium bicarbonate but readily soluble in caustic soda. This compound, which has the formula $C_{19}H_{18}O_8Cl_2$, apparently results from the opening of a depside link with esterification of the resulting carboxyl group.

From the above it is surmised that Gangaleoidin is a Depside of the diphenyloxide type with substituents as illustrated below.



It is apparently closely related to Diploicin isolated by one of us from the lichen *Buellia canescens*, the reactions of Diploicin being at present best explained by the following formula:—



Diploicin contains a methoxyl group which can only be demethylated with extreme difficulty.

Experimental.

220 g. of air-dried lichen were ground and extracted with ether. The ether on evaporation left a residue (18·7 g.) of golden brown flakes which possessed an odour not unlike that of quinone. This material was warmed with 50 c.c. ligroin, which removed some red oily material. The portion insoluble in ligroin was warmed with two successive amounts (50 c.c.) of benzene. The benzene liquors on cooling deposited crystals, which on being twice recrystallised from benzene melted at 194·5° C. to 196·7° C. On evaporation of the benzene mother liquors, and treatment with ligroin, a soluble and an insoluble fraction were obtained. The former melted about 220° C., but was impure; the latter was found to consist principally of Gangaleoidin (*vide infra*).

The portion of the ether-extract remaining after treatment with ligroin and benzene, as indicated above, was warmed with 50 c.c. alcohol, and filtered. The alcoholic liquors on cooling deposited a small amount of brownish material, which on being crystallised twice from benzene gave white needles of

[1] Scientific Proceedings R.D.S., 21, p. 67, 1934.

Gangaleoidin m.p. 213–214° C. The fraction insoluble in alcohol was dissolved in 70 c.c. acetone, and on allowing the solution to stand crystals were deposited, which, on precipitation by dissolving in acetone and adding an equal volume of alcohol, were obtained as snow-white needles m.p. 214–215° C. (Fig. 1). This material we call Gangaleoidin. In the impure form it tends to turn pink in the air.

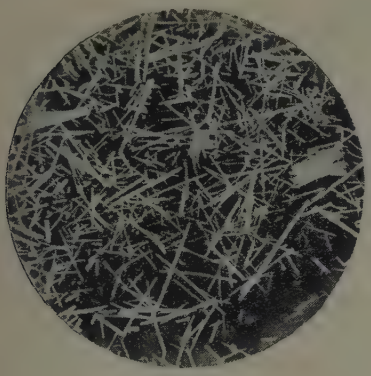


FIG. 1.

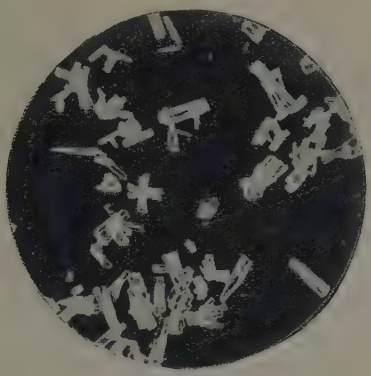


FIG. 2.

Analysis of Gangaleoidin.

All analyses were carried out on material dried at 110° C. in high vacuum over P_2O_5. Results are by Dr. A. Schoeller. Sample A was crystallised from acetone-alcohol; sample B from benzene.

	Wt. of Substance	CO_2	H_2O	Percentage C	Percentage H	Percentage Cl
A.	5·012 mg.	9·640 mg.	1·600 mg.	52·47	3·55	—
B.	4·591 „	8·810 „	1·430 „	52·27	3·45	—
	2·993 „ gave 0·52 mg. Cl.			—	—	17·38
	3·370 „ „ 0·576 „ „			—	—	17·07
	3·550 „ „ 4·075 mg. Ag I. OCH_3 = 15·1%.					
	0·293 mg. in 3·085 mg. camphor 9·5 Δ M.W. = 399					
	0·416 „ „ 3·930 „ „ 10·5 Δ M.W. = 405					

$C_{16}H_8O_5Cl_2(OCH_3)_2$ requires C = 52·3; H = 3·39: Cl = 17·19: OCH_3 = 15·0. M.W. = 413.

Properties of Gangaleoidin.

Gangaleoidin is sparingly soluble in methyl and ethyl alcohols, cold benzene, ether, and chloroform. It dissolves easily in warm benzene and acetone. It dissolves slowly in 10 per cent. aqueous potash, complete solution being effected

after 3 days. The crystals give no colour reaction with bleaching powder solution, and are stained a faint yellow colour by concentrated sulphuric acid, in which they dissolve to a colourless solution. An alcoholic solution shows no colour reaction with ferric chloride.

Gangaleoidin Acetate.

(1) 0·2 g. of Gangaleoidin was added to 10 c.c. acetic anhydride, and a few drops of concentrated sulphuric acid were added. The greater part of the material went into solution, and after 15 minutes a solid was deposited. The whole was poured into 40 c.c. water. The solid which was precipitated was well washed with water, dried, and crystallised from a mixture of 5 c.c. chloroform and 10 c.c. alcohol, from which it separated as short prisms m.p. 244–245° C. (Fig. 2).

(2) 0·2 g. of Gangaleoidin 6 c.c. acetic anhydride and 0·6 g. of sodium acetate were boiled for 15 minutes. The mixture was then cooled and poured into 25 c.c. water. The white solid thus obtained was washed with water, dried, and crystallised from a mixture of 5 c.c. chloroform and 5 c.c. alcohol. The product thus obtained had m.p. 245–246° C. Mixed with the crystals obtained in (1) there was no depression in melting point.

Analysis of Gangaleoidin Acetate.

	Wt. of Substance	CO_2	H_2O	Percentage C	Percentage H	Percentage Cl
(1)	4·797 mg.	9·300 mg.	1·550 mg.	52·93	3·59	—
(2)	4·353 „	8·390 „	1·400 „	52 56	3·56	—
	2 750 „ gave	0·477 „ Cl.		—	—	16·26
	3·797 „ „	3·660 mg. Ag I.	$OCH_3 = 12·74$			

$C_{16}H_7O_5Cl_2(CH_3CO) . (OCH_3)_2$ requires C = 52·75 : H = 3·52 : Cl = 15·6.
$OCH_3 = 13·6$.

Action of methyl alcoholic potash on Gangaleoidin.

0·25 g. of Gangaleoidin was dissolved in 25 c.c. methyl alcohol, and to the warm solution 5 c.c. of 5 per cent. methyl alcoholic potash was added. The mixture was gently warmed for 1 minute, and on testing a small portion of the solution it gave no precipitate on dilution with water. The main bulk was poured into 20 c.c. water, filtered from a small amount of flocculent precipitate, and acidified. On standing a mass of fine needles was precipitated. This was filtered off, dried, and crystallised from 10 c.c. benzene, from which it separated as rectangular prisms m.p. 198·5° C.

The product is insoluble in 3 per cent. sodium bicarbonate, but readily soluble in 2 per cent. caustic soda. It dissolves in sulphuric acid to a colourless

solution. A solution of the product in alcohol gives no colouration with ferric chloride.

			Percentage		
Wt. of Substance	CO_2	H_2O	C	H	Cl
4·947 mg.	9·310	1·840	51·33	4·13	—
3·415 „ gave 0·563 mg. Cl.			—	—	16·48
3·573 „ „ 5·470 mg. Ag I. OCH_3 = 20·2.					

$C_{16}H_9O_5Cl_2(OCH_3)_3$ requires C = 51·2 : H = 4·04 : Cl = 15·96 : OCH_3 = 20·9.

Extraction of the Lichen with Acetone and with Chloroform.

When the lichen had been extracted with ether as indicated above, it was air-dried and extracted further with acetone. The acetone extract was evaporated to dryness, treated with 10 c.c. warm benzene, and then with 10 c.c. alcohol. The residue insoluble in these solvents was dissolved in acetone, and filtered from a small amount of dark material. On standing, the acetone solution deposited crystals, m.p. 196·5° C., identical with the material of similar melting point obtained from the ether extract. After extraction with acetone the lichen was further extracted with chloroform, but this yielded only 0·4 g. of a sticky material, which was purified by washing with alcohol, and then crystallising from a mixture of choloroform and alcohol. This gave a small amount of the product of m.p. 196·5° C.

This product is sparingly soluble in alcohol and acetone, but dissolves in warm benzene. It is soluble in 10 per cent. caustic potash to form a yellow solution, which on acidification with dilute hydrochloric acid gives a white precipitate. It is insoluble in 3 per cent. sodium bicarbonate. An alcoholic solution of the material gives with ferric chloride a port-wine colouration. It crystallises in the characteristic form of Atranorin. Further quantities of this material are being prepared for the purpose of closer characterisation.

3·483 mg. substance gave 0·242 mg. Cl, corresponding to 6·1 per cent. Cl.

No. 15.

THE CELLULOSE OF MARINE ALGAE.

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When Stanford (1) extracted alginic acid from seaweed by means of a solution of sodium carbonate, he obtained an insoluble residue, from which he was able to make a transparent paper, and to which he gave the name "algic cellulose." That this material was identical with, or contained cellulose of the same chemical constitution as, the cellulose of land plants was tacitly assumed by most chemists and botanists, and Kylin (2) showed that the cell-wall constituents of various seaweeds gave with iodine and sulphuric acid the characteristic blue colour of cellulose.

Nevertheless, some doubt seems to exist as to the occurrence of normal cellulose in marine algae. Thus, Atsuki and Tomoda (3) state that normal cellulose is not to be found in the crude fibre of *Laminaria saccharina*; while, in the algae examined by him, Ricard (4) did not obtain the characteristic reaction of cellulose as found by Kylin. Furthermore, Ricard, and subsequently Gomez (5), failed to obtain sugars by the hydrolysis of the fibre of various marine algae. On the other hand, Naylor and Russell-Wells (6) have recently demonstrated the existence in several varieties of red algae of a substance which dissolves in cuprammonia, and which behaves towards acetylating agents like normal cellulose.

In view of these somewhat conflicting views, the results of our experiments on the fibre of *laminariae* should be of interest. As already briefly announced in "Nature" (7), we isolated from this variety a substance from which we obtained glucose by hydrolysis, and which, with carbon disulphide, formed a viscose resembling the viscose from ordinary cellulose. We also obtained from it acetylated and methylated compounds with properties similar to those of the corresponding derivatives of ordinary cellulose.

Our interest in the fibrous portion of marine algae first arose from the discovery of Nelson and Cretcher (8) that alginic acid is a polymerised derivative of mannuronic acid. In view of this fact, as well as the well-known

occurrence of mannitol in the algae, we thought it possible that "algic cellulose," which in physical properties is somewhat different from the cellulose of land plants, might be a polymer of mannose. As a matter of fact, though we obtained from the products of hydrolysis large quantities of glucosazone, we found no trace of the insoluble phenylhydrazone of mannose. Thus it is definitely established that the marine algae contain true cellulose of the same chemical character as the cellulose of land plants. Our experiments were carried out only with *Laminaria digitata*, but it is not too much to assume that they hold for other varieties of brown algae.

Failure to obtain the reactions of cellulose with the crude fibre from algae, and failure to obtain glucose by its hydrolysis, are probably to be attributed to the impurity of the cellulose used. We have found that the latter substance is, in the crude fibre, chemically combined or at least closely associated with a body which can only be removed by comparatively drastic treatment. We have not yet obtained any exact information as to the chemical composition of this body; but in its close association with the cell-wall material it certainly resembles the lignin of land plants. When the alginic acid is removed from the plant by means of sodium carbonate, the fibrous residue is disintegrated, and the presence of alkali-soluble bodies other than alginic acid may not be noticed. If, however, the alginic acid is removed by means of ammonia, as recommended by Schmidt and Vocke (9), the presence of the lignin-like body at once becomes obvious. Repeated extractions with ammonia remove all the alginic acid without destroying the shape of the fronds or stipes. Boiling dilute hydrochloric acid will now remove acid-soluble bodies in small amount, and when the residue from this treatment is boiled with caustic soda of from five to ten per cent. strength the lignin substance is dissolved out.

The above is a suitable method for isolating the cellulose. In our experiments, however, it was frequently replaced by a retting process which is more convenient for obtaining a large supply, especially where wet seaweed is available. When the latter is kept in a vessel exposed to the weather it undergoes fermentation, yielding a liquid which contains acetic and other organic acids as well as inorganic salts (10). Even after twelve months the shape of the plant is still preserved; but if after the lapse of about a month the material is handled it will be found to be extremely soft, and will disintegrate easily. If it is now extracted with ammonia little or no alginic acid will be obtained; but, on extraction with hot caustic soda, the lignin substance is dissolved out, as before, leaving the cellulose.

Experimental.

Preparation of Cellulose from Dried Seaweed.—About 100 g. of dried laminaria fronds were steeped in dilute (1 per cent.) hydrochloric acid, washed with water, covered with dilute ammonia, and left standing overnight. The algin solution was strained off through a Buchner funnel (without filter paper), and the solid residue was again covered with ammonia and left standing as

before. This extraction was repeated until the ammoniacal solution gave only a slight precipitate on acidulation. The solid matter was now boiled with dilute hydrochloric acid, filtered through cloth, and washed with water. The acid extracted only a small quantity of substance. The shape of the leaves was still apparent in the solid, though it had been disintegrated into small pieces. It was treated with an excess of 5 per cent. caustic soda, boiled for a few minutes, and filtered through a glass filter. This boiling with caustic soda was repeated 9 times. After 2 or 3 boilings the material was completely disintegrated, and appeared in the form of a black powder. After further boilings streaks of white began to appear in the cake of filtered solid, which by this time had diminished considerably in bulk. After the ninth boiling it was brownish-white. It was now bleached with a solution of calcium hypochlorite and hydrochloric acid, washed with water containing a little sodium carbonate, and then with distilled water. Finally, on washing with alcohol and ether and drying in the steam oven, the cellulose was obtained in the form of a cream-coloured powder.

Preparation of Cellulose from Retted Seaweed.—A quantity of *Laminaria digitata* was left in a tank exposed to the weather. After some months some of the fronds were removed, washed with running water, boiled for an hour with dilute hydrochloric acid, and filtered. Excess of hydrochloric acid was next removed by washing well with water, and the material was boiled for an hour with 9 per cent. caustic soda. During the boiling the colour darkened considerably, and the fronds became disintegrated. Three successive boilings with the soda solution sufficed to remove the lignin substance, leaving the cellulose as a greenish coloured mass. It was suspended in water and bleached with a current of chlorine gas. The resulting slate-coloured product was again boiled with caustic soda, by which only a small amount of substance precipitable by acids was dissolved. On washing, first with water and then with alcohol and ether, and drying, the cellulose was obtained as a greyish powder.

The material obtained by either of these methods slowly develops a blue colour with iodine and sulphuric acid, and dissolves in Schweitzer's reagent. It was this purified cellulose that was used in the experiments described below.

Acetylation of Algic Cellulose.—This acetylation was carried out by the method of Ost described by Heuser (11). 9 g. of algic cellulose were added in four portions to a solution of 9 g. zinc chloride in 36 c.c. acetic acid. Into the stirred mixture 36 c.c. of acetic anhydride were slowly dropped, the addition being spread over 4 hours. No change was apparent while the mixture was cool, but on warming a syrup was obtained which still, however, contained some lumps of solid. The syrup was thinned by addition of acetic acid, and stirred to disintegrate the lumps. The whole product was poured into water, and the precipitated acetate was washed repeatedly with water. As the acetic anhydride could not be removed from the lumps by washing, these were picked out, dried with alcohol and ether, and ground to a granular mass in a mortar. This was now freed from acetic anhydride by boiling with water. The product was completely soluble in chloroform.

Preparation of Viscose.—In this preparation we followed the well-known method of Cross, Bevan, and Beadle (12). 30 g. of moist algic cellulose, containing about 5 g. of dry matter, were treated with 90 c.c. of 18·5 per cent. caustic soda, and left standing for 5 hours. At the end of that time excess of soda was removed by filtering through linen, and the alkali cellulose was disintegrated as much as possible by hand. It was transferred to a flask, treated with 12 c.c. of carbon disulphide, corked, and allowed to stand for 12 hours with frequent shaking. The contents of the flask changed in colour through yellow to brown. Excess carbon disulphide was poured off, and the xanthate was stirred briskly with dilute caustic soda for an hour and a half. It dissolved completely in the soda. The cellulose was precipitated with strong hydrochloric acid as a greyish mass. It was filtered, washed with water, alcohol, and ether, and dried. The product consisted of 4·5 g. of a greyish fibrous body.

Methylation of Algic Cellulose.—The method used was that of Urban (13). 9 g. of moist algic cellulose, containing about 1 g. of dry matter, were stirred briskly with 100 c.c. of 45 per cent. caustic soda for a few minutes, during which time 10 c.c. of acetone were added. 100 c.c. of methyl sulphate were now dropped into the stirred mixture during 10 hours continuously, the reaction flask being cooled with running water. The product, which had become somewhat lumpy, was filtered, and washed with cold water. It was submitted twice again to the same methylation process. After the third methylation it was washed with water, alcohol, and ether, and left standing in chloroform for some hours. It dissolved completely, giving a highly viscous solution.

Hydrolysis of Algic Cellulose.—Willstatter and Zechmeister (14) showed that cellulose dissolves in 40 per cent. hydrochloric acid, undergoing hydrolysis on standing, and finally being completely converted into glucose. We found that dry algic cellulose also dissolves rapidly in this reagent. Like ordinary cellulose it is reprecipitated if the solution is diluted soon after its formation; but if left standing it rapidly undergoes hydrolysis, and within an hour from the time of going into solution no precipitate is obtained on pouring into water.

Notwithstanding the ease with which the cellulose is hydrolysed by the Willstatter-Zechmeister process, we found it more convenient for the purpose of identifying the sugar to bring about the hydrolysis with 72 per cent. sulphuric acid following the method of Ost (15). 8 g. of moist cellulose, containing 2 g. of dry matter, were put into a flask with 20 c.c. of 72 per cent. sulphuric acid (allowance being made for the water-content of the cellulose in making up the acid). After the lapse of 48 hours most of the material had gone into solution, leaving a small amount of dark-coloured residue. The mixture was diluted to 800 c.c. with water, filtered, and heated in an autoclave to 120° C. for an hour. It was now neutralised with lead carbonate, filtered, and evaporated to small bulk under reduced pressure. When this (light yellow) solution was warmed on the water bath with phenylhydrazine acetate an osazone was rapidly formed in large quantities. This was dissolved in absolute alcohol, and precipitated with water, and then dissolved in pyridine, and precipitated with water. It now melted at 200–201° C.

The most characteristic reaction of mannose is the formation of the insoluble phenylhydrazone (16). The hydrolysed algic cellulose gave no immediate precipitate with phenylhydrazine acetate in the cold, though on standing for 24 hours yellow needle-shaped crystals separated out. This body was not, however, mannose phenylhydrazone as comparative tests showed. A sample of mannose phenylhydrazone prepared by us melted at 187° C. It was insoluble in acetone, and colourless. The above yellow crystals melted at 196–197° C., which is close to the melting point given for the phenylhydrazone of l-mannose; but they were easily soluble in acetone.

The melting point of glucosazone appears to vary considerably with its treatment, possibly owing to a variation in the relative quantities of α and β osazones present. A sample of osazone prepared from pure glucose, on recrystallisation from acetone, melted at 201–202° C., which is close to the melting point of the osazone obtained by heating the hydrolysed algic cellulose with phenylhydrazine. On the other hand, some of the same glucosazone dissolved in alcohol, and precipitated with water, melted at 195–197° C. A mixture of this with the crystals slowly deposited in the cold melted at 194–197° C.

From these experiments it seems that the body melting at 201–202° C., obtained by heating the product of hydrolysis with phenylhydrazine acetate, and the body melting at 196–196·4° C., obtained by the slow action of phenylhydrazine in the cold, were both glucosazone, and in fact that the only product of complete hydrolysis of algic cellulose is glucose.

NOTE.—In a recent paper, Challinor, Haworth, and Hirst (17) state that "it does not seem to be generally known that fructose yields an osazone without heating in the presence of phenylhydrazine." This remark suggests the possibility that the osazone obtained from the hydrolysed algic cellulose in the cold originated from traces of a polysaccharide of the levan type in the cellulose. We found, however, that filter paper and raw cotton hydrolysed in the same way as algic cellulose also slowly formed the osazone in the cold. Furthermore 5 c.c. of a 4 per cent. (approximately) solution of pure glucose deposited about 0·02 g. of impure osazone in 24 hours. This represents a 7 per cent. yield of osazone. These experiments show that the *slow* deposition of glucosazone from the hydrolysed cellulose in the cold cannot be taken as conclusive evidence of the presence of fructose in the solution.

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No. 16.

THE THERMAL DECOMPOSITION OF HYDROGEN PEROXIDE IN PRESENCE OF GLASS WOOL AND COPPER SULPHATE.

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It has long been known that the decomposition of hydrogen peroxide may be accelerated by the presence of a glass surface. Elissafoff (Z. Elektrochem., **21**, 354, 1915) re-examined the question, using two varieties of commercial hydrogen peroxide (medicinal, and Merck's perhydrol) and found, in agreement with other workers, that the reaction is of the first order.

The introduction of 0·5 g. glass wool into 20 c.c. solution (12 millimolar) caused a marked increase in the rate of decomposition, the first order velocity constant at 25·5° being multiplied by 1·3 for Jena glass wool, 3·7 for Kahlbaum glass wool, and 13 for Russian glass wool. These differences Elissafoff connected, no doubt correctly, with the greater readiness with which the Kahlbaum and Russian glass wool yielded alkali to water, the acceleration of peroxide decomposition by alkali being well known, but was of the opinion that there was a genuine heterogeneous catalysis at the surface of the glass wool.

The most important part of Elissafoff's paper concerned the effect of copper and manganese sulphates, both of which act as catalysts of decomposition. The glass wool and metallic sulphate together caused an increase in the rate of peroxide decomposition which was very much greater than would be expected on an additive basis. For example, at 25·5°, the velocity constant of peroxide decomposition in presence of glass wool was 0·06, of copper sulphate (1·54 millimoles per litre) 0·10, and of both 0·67.

Elissafoff showed that, while the velocity constants in two experiments in which the same weights of Jena glass wool were present and copper sulphate concentrations were 1 and 10 millimolar, respectively, were in the ratio 1 : 1·77, the amounts of copper adsorbed by Jena glass powder from similar copper solutions were in the ratio 1 : 1·73. He concluded that the great increase in catalytic effect when both copper sulphate and glass wool are present is due to the adsorption of the copper on the surface of the glass, where it is particularly effective owing to its increased concentration at what is presumably already the chief site of reaction.

Elissafoff's work has received considerable prominence, and appears in many books dealing with catalysis as one of the examples of promoter action in which the mechanism is clear. For instance, Rideal and Taylor ("Catalysis in Theory and Practice," p. 104, 1926) preface their account of this investigation with the words, "One case in which the mechanism of promoter action seems definitely to have been ascertained is available in the older literature." This fact gives importance to a more recent communication by Robertson (J. Amer. Chem. Soc., **52**, 382, 1931) in which doubt is cast on the validity of Elissafoff's conclusions. Robertson did obtain an increase in decomposition velocity on addition of glass wool to a solution of hydrogen peroxide containing copper sulphate, but less than that observed by Elissafoff. He found that a filtered extract of glass wool catalysed the decomposition as much as the original glass wool, and that increasing the amount of glass wool tenfold made very little difference to the velocity, results which are hard to reconcile with the view that the catalytic effect of glass wool is mainly a surface phenomenon. Robertson believes that copper is adsorbed on the surface of the glass wool as a basic salt, which is further oxidised to copper peroxide, the reaction being favoured by the alkali from the glass. His paper, which is very brief, gives no detailed experimental results, but he mentions that reaction rates were not easily reproduced. The source of his hydrogen peroxide is not stated.

A third investigation which bears directly on the problem is that of Spitalski, Petin, and Konovalova (J. Russ. Phys. Chem. Soc., **60**, 1237, 1928) and Spitalski and Konovalova (*ibid.*, **62**, 1033, 1930) (available to the writer in abstracts only) although in their experiments no glass wool was used. They formed the opinion that copper ions have little or no effect, copper scarcely catalysing the decomposition in acid solution, and that the true catalyst, at p_H's at which precipitation occurs, is copper hydroxide, with a copper peroxide acting as intermediate compound.

In view of the conflict in experimental results, as well as in theory, between these pieces of work it seemed advisable to re-investigate the reaction.

Elissafoff's work was done with commercial hydrogen peroxide and Robertson's with peroxide of unspecified origin. In order to eliminate unrecognised effects due to stabilisers such as are usually present in commercial peroxide (mineral acids, barbituric acid, acetanilide, etc.) the experiments now described were performed with pure solutions of hydrogen peroxide. Various methods of preparation were tried, that eventually adopted being that of Kilpatrick, Reiff, and Rice (J. Amer. Chem. Soc., **48**, 3019, 1926) from sodium peroxide and sulphuric acid.

It was found in passing that, when the resulting mixture was distilled under reduced pressure, only a very dilute solution of peroxide was obtained, the bulk of the peroxide having attached itself to the sodium sulphate as hydrogen peroxide of crystallisation. When the solid residue was heated concentrated peroxide distilled over. An analysis showed that the crystals which separated from the concentrated solution had the composition

$Na_2SO_4 . 9H_2O . H_2O_2$ (described by Tanatar, Z., anorg. Chem., **28**, 256, 1901). A few experiments were performed in which sodium peroxide was added to a solution containing 20 per cent. sulphuric acid and 30 per cent. hydrogen peroxide, in the hope of preparing sodium sulphate crystals in which more than one molecule of water had been replaced by hydrogen peroxide. The attempt was unsuccessful.

Except for a few early trials which gave unsatisfactory results, all experiments were carried out in quartz flasks, covered with black cloth to exclude complications from the photochemical decomposition, and immersed in a thermostat at 35° C. 5 c.c. samples were removed from time to time, decomposition was arrested by diluting with cold water acidified with sulphuric acid, and the samples were titrated with N/100 $KMnO_4$.

It seemed reasonable, before examining the reaction on glass wool, with or without added copper, to determine the decomposition velocity of pure hydrogen peroxide in quartz at the temperature of the experiments. The difficulty of getting consistent results in such experiments has been noted by many workers, including Lemoine (J. Chim. Phys., **12**, 1, 1914) and Robertson (*loc. cit.*). After obtaining many divergent results from experiments in which no intentional variation had been made, the writer was driven to accept the conclusion of Rice (J. Amer. Chem. Soc., **48**, 2099, 1926) that dust-free hydrogen peroxide decomposes extremely slowly and that, therefore, the rate of decomposition of supposedly pure hydrogen peroxide may have very varying values, depending on the number of invisible catalytic particles accidentally introduced, and with a lower limit fixed by the surface of the containing vessel.

Dust was removed in Rice's experiments by a process of repeated slow distillation without ebullition, but the writer found that peroxide could be rendered sufficiently dust-free to show only very slow decomposition if it were filtered through several layers of a very fine filter-paper (Whatman, No. 44) into a quartz or pyrex glass flask which had been repeatedly washed with similarly filtered distilled water. This immunity from rapid decomposition was noted even in cases where the peroxide had been rendered distinctly alkaline with sodium hydroxide before filtration (p_H after filtration, 8·9), the hydroxyl ions being apparently unable to exert their usual catalytic effect in absence of the solid particles.

It was assumed, therefore, that in comparison with the catalytic decomposition under examination, the thermal decomposition of hydrogen peroxide in the absence of solid surfaces, even in the presence of hydroxyl ions, was negligibly slow.

A.—*Decomposition in presence of Glass Wool.*

The glass wool used, on contact with distilled water (and more rapidly on boiling), rendered the solution alkaline. The surface was freed from adventitious impurities (particularly traces of iron rust) by treating several times with dilute

hydrochloric acid, and then washing with distilled water until no further trace of acid could be extracted. Large batches of wool were thus treated to secure uniformity, and the washed and dried wool stored in stoppered bottles.

In the following experiments, the quartz reaction flask was well washed with doubly-filtered distilled water, a suitable weight of glass wool was weighed into it (0·2 to 0·7 g.), 50 c.c. H_2O_2 (approximately 2·5 N/100), with or without the addition of a quantity (from 1 to 3 c.c.) of N/100 NaOH, were doubly filtered into the flask, and the flask loosely stoppered and placed in the thermostat at 35°. After an interval to allow temperature equilibrium to be established, 5 c.c. samples were withdrawn periodically and titrated, and the p_H of 2 c.c. samples was determined by addition of two drops of B.D.H. mixed indicator and comparison with the colours of buffer solutions similarly treated.

Each experiment lasted two or three days. It was found that the p_H of the solution increased over a period of 3 to 10 hours, and then remained fairly constant or fell slowly.

As the decomposition of hydrogen peroxide is usually stated to be a reaction of the first order, unimolecular velocity constants (K_1) were calculated by the equation

$$K_1 = \frac{2{\cdot}303}{t_2 - t_1} \cdot \log_{10} \frac{a - x_1}{a - x_2},$$

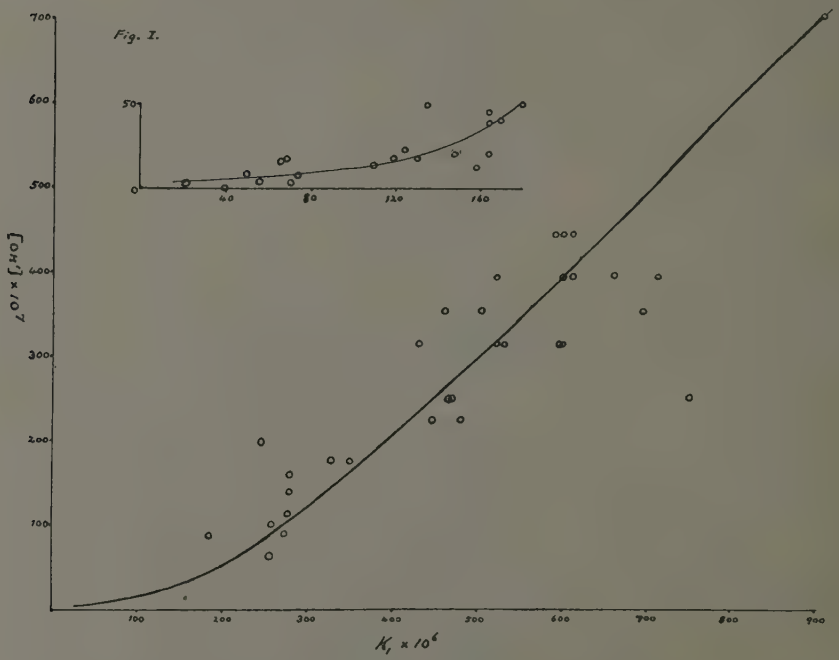
where a represents the original concentration of hydrogen peroxide, and $(a - x_1)$ and $(a - x_2)$ the concentrations at times t_1 and t_2 respectively. Times are measured in minutes, and concentrations of peroxide by the number of c.c. N/100 $KMnO_4$ required to oxidise 5 c.c. of solution. It was found that the values of K_1 obtained for a given p_H were fairly constant, whether the p_H had been attained by the use of a large quantity of glass wool without added sodium hydroxide, or by the use of a small quantity of glass wool with the addition of sodium hydroxide. The experimental error throughout is large, but this is not surprising, in view of the sensitiveness of the reaction to traces of impurity and the impossibility of ensuring that the glass wool had been freed from such impurities in every case to the same extent. The average p_H values recorded can only be regarded as approximate, as p_H observations were made only when the flask was opened to remove a sample for titration. Frequent opening of the flask is undesirable, as it increases the likelihood of accidental contamination.

In Table 1 are recorded the results of 13 experiments, the last series of this type carried out. The results of a very large number of earlier experiments were eventually rejected, owing to the gradual discovery of various sources of error, some of which have been mentioned.

TABLE 1.

Expt. No.	Wt. of glass wool (g.).	C.c. of N/100 NaOH.	Time interval (min.).	$[OH'_7]$ $\times 10^7$.	$K_1 \times 10^6$.	K_2,	Average peroxide conc.
136a	0·2	–	0– 263	1·6	25	156	12·22
b			263–1084	2·5	56	220	11·90
c			1084–3958	2·8	71	254	10·57
137a	0·2	3	0–1084	50	180	36·0	11·15
b			1084–2570	40	170	42·5	8·96
c			2570–3958	13	110	84·6	7·28
132a	0·3	–	0– 181	8	50	62·6	12·22
b			181– 376	18	69	38·3	12·09
c			376– 658	22	124	56·4	11·80
d			658–1777	20	148	74·0	10·70
133a	0·3	1	0– 171	7	74	106	12·76
b			171– 648	18	120	66·7	12·33
c			648–1461	18	130	72·2	11·39
d			1461–1773	11	158	144	10·51
e			1773–2849	20	164	82·0	9·44
134a	0·3	2	0– 509	79	164	20·7	11·36
b			509–1265	158	279	17·7	9·85
c			1265–1445	141	278	19·7	8·60
d			1445–3038	100	257	25·7	6·97
135a	0·3	3	0– 190	251	465	18·5	11·53
b			190– 505	316	597	18·9	10·07
c			505–1261	355	697	19·6	7·26
128a	0·5	–	0– 63	16	66	41·2	12·27
b			63– 190	89	181	20·4	12·11
c			190–1308	178	325	18·4	10·37
d			1308–2642	355	460	13·0	6·63
130a	0·5	1	0– 209	50	135	27·0	11·89
b			209– 442	112	272	24·3	11·36
c			442–1310	178	349	19·6	9·50
d			1310–2820	316	429	13·6	6·10
e			2820–4147	355	503	14·2	3·17
129a	0·5	2	47–1151	251	464	18·5	9·84
b			1151–1302	398	602	15·1	7·05
c			1302–1735	398	521	13·1	6·05
d			1735–2648	398	660	16·6	4·15
131a	0·5	3	0– 209	251	750	29·9	11·05
b			209– 439	316	602	19·0	9·55
c			439–1307	447	612	13·7	6·99
d			1307–2816	316	530	16·8	3·69
e			2816–4142	224	445	19·9	1·78
139a	0·6	1	0– 174	45	163	35·6	12·36
b			174– 563	90	265	29·4	11·59
c			563–1426	200	243	12·2	9·95
d			1426–3060	447	590	13·2	6·15
140a	0·7	–	0– 150	63	252	40·0	11·83
b			150– 843	224	478	21·3	9·97
c			843–1194	316	520	16·5	7·64
d			1194–2328	398	710	17·8	5·10
141a	0·7	1	0– 142	89	415	46·6	12·15
b			142–1186	398	610	15·3	9·01
c			1186–2320	708	910	12·9	4·30

The dependence of K_1 on the hydroxyl ion concentration is clear from Fig. 1, and a reference to Table 1 will show that, where several values are recorded for the same hydrogen ion concentration, the highest value is not necessarily that of the experiment with the largest amount of glass wool. There is, indeed, nothing to suggest that the amount of glass wool is a factor of much importance. Even a small amount apparently provides an adequate surface, and the comparative constancy of the results obtainable in such experiments for a given p_H is in striking contrast with the variations obtained in the experiments mentioned earlier where the only surfaces available were those of the vessel and of adventitious dust particles.



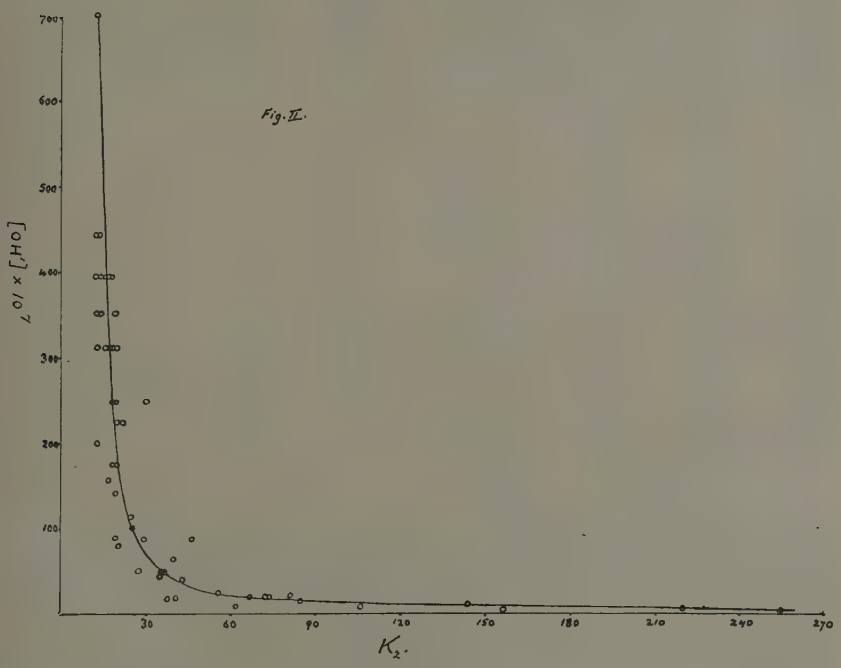
Some experiments were performed with a different batch of glass wool which yielded alkali much less freely to the solution. It was found that the velocities obtained fitted quite well into the curve of Fig. 1, although much greater amounts of glass wool had to be used to obtain the same hydroxyl ion concentration.

The calculation of a bimolecular constant (K_2) by substituting $v = K_2[H_2O_2]\,[OH']$ for $v = K_1[H_2O_2]$ yields interesting results. $K_2 = K_1/[OH']$, the hydroxyl ion concentration used being the average concentration over the period concerned.

It is found that K_2 is fairly constant over the range $[OH'] \times 10^7 = 150$ to 700 (this covering a wide range of peroxide concentrations). At lower hydroxyl ion concentrations, however, the values of K_2 increased enormously (Fig. 2).

The form of the curve in Fig. 2 suggests that, while at high hydroxyl ion concentrations the reaction velocity may be fairly accurately represented by the equation $v = K_2[H_2O_2]\,[OH']$, at low hydroxyl ion concentrations a second kind of decomposition which is independent of hydroxyl ions becomes of importance, and that we have probably decompositions of the first and second orders proceeding simultaneously and independently on the surface of the glass wool, the whole being represented by the equation

$$v = K_2[H_2O_2]\,[OH'] + K_1[H_2O_2].$$



The results obtained by making $K_2 = 15$ and $K_1 = 7 \times 10^{-5}$ are shown in Table 2, in which the experimental results are arranged in order of increasing value of theoretical velocity (v_t) as calculated from the above equation, v_e being the approximate experimental velocity under the conditions set forth in Table 1. If experiment and theory were in perfect accord, the value of v_e / v_t would be unity in every case. As it is, 45 per cent. of the values of this ratio lie between 0·87 and 1·15, and 75 per cent. between 0·75 and 1·30. No

satisfactory way of representing the results graphically was discovered, but a careful examination of Table 2, with the help of the data of Table 1, shows that the departures from the theoretical are quite haphazard, and such as can reasonably be attributed to the unavoidably large experimental error.

TABLE 2.

Expt. No.	$v_t \times 10^3$.	$v_e \times 10^3$.	v_e/v_t.	Expt. No.	$v_t \times 10^3$.	$v_e \times 10^3$.	v_e / v_t.
137c	0·65	0·80	1·23	128b	2·47	2·20	0·89
131e	0·72	0·80	1·11	141a	2·48	5·40	2·18
133d	0·75	1·61	2·15	130b	2·71	3·10	1·14
136c	0·78	0·74	0·95	129d	2·77	2·70	0·98
136b	0·88	0·68	0·77	134b	3·02	2·70	0·90
136a	0·89	0·31	0·35	130c	3·21	3·40	1·06
133e	0·94	1·54	1·64	130d	3·32	2·50	0·75
132a	1·00	0·80	0·80	140d	3·40	3·30	0·97
133a	1·03	1·00	0·97	128c	3·50	2·90	0·83
132d	1·07	1·52	1·42	139c	3·67	2·45	0·67
133c	1·10	1·55	1·41	129c	4·04	3·10	0·77
132b	1·15	0·84	0·73	140b	4·05	4·60	1·14
128a	1·15	0·80	0·70	128d	4·11	3·20	0·78
137b	1·17	1·50	1·28	140c	4·16	4·05	0·98
133b	1·19	1·29	1·08	135c	4·38	4·90	1·12
132c	1·22	1·40	1·14	129a	4·39	4·50	1·02
134d	1·53	1·80	1·18	139d	4·55	3·60	0·79
137a	1·62	2·00	1·23	129b	4·82	4·20	0·87
139a	1·70	2·25	1·32	141c	4·87	3·70	0·76
130a	1·72	1·70	0·99	131a	4·91	8·00	1·63
130e	1·91	1·50	0·79	135a	5·14	5·40	1·05
140a	1·95	3·60	1·85	131c	5·18	4·40	0·85
131d	2·01	1·90	0·95	131b	5·20	5·70	1·10
134a	2·15	1·90	0·88	135b	5·48	6·00	1·10
139b	2·36	3·10	1·31	141b	6·17	5·20	0·84
134c	2·41	2·40	1·00				

B.—*Decomposition in presence of Glass Wool and Copper Sulphate.*

A number of experiments was performed in which glass wool, copper sulphate solution, and hydrogen peroxide solution were mixed in a quartz flask, and the decomposition of hydrogen peroxide and changes in p_H followed as before. An attempt was also made to estimate the distribution of copper between solution and glass wool. The results were not very concordant, and the following method was eventually adopted :—

Two solutions of copper sulphate were prepared, one containing 0·0000194 g. copper per c.c., and the other 0·000194 g. per c.c. These solutions are said to contain 1 and 10 parts of Cu per c.c. respectively, 0·0000194 g. Cu being styled a "part" throughout. It was found that if 0·5 g. of glass wool were allowed to stand overnight in 30 c.c. of copper sulphate solution, made up from the

foregoing solutions and water, if the solution contained less than about 40 parts of copper the glass wool adsorbed practically all the copper. If the concentration of copper was further increased very little more copper was adsorbed. Similar results were obtained with smaller weights of glass wool.

The copper appeared to be taken up mainly as oxide or hydroxide (little or no $-SO_4$ being removed from solution), and was sufficiently firmly adsorbed for none of it to be removed by washing with water. Dilute acetic acid removed it rapidly. The glass wool with its adsorbed copper was greenish in colour, but, when the wool was treated with hydrogen peroxide, the colour rapidly changed to a brownish-yellow, the copper hydroxide being presumably altered to copper peroxide which was probably an "intermediate compound" in the decomposition of the hydrogen peroxide.

The coppered glass wool was squeezed with a glass rod until the greater part of the washing water had been removed. It was then transferred to a quartz flask, and the filtered peroxide solution (with or without additional copper sulphate or sodium hydroxide) added. The estimations were carried out as before. Decomposition of the peroxide was now much more rapid, and followed quite a different law from that in presence of glass wool alone.

TABLE 3.

Expt. 164. 0·3 g. glass wool, with 26 parts of adsorbed copper.

Time interval (min.) .	0–15	15–30	30–48	48–78	78–106	106–124	124–134
Avge. $[H_2O_2]$ . .	11·91	10·73	9·30	6·79	4·16	2·46	1·52
P_H . . .	7·3	7·3	7·2	7·2	7·1	7·1	7.1
$K_1 \times 10^3$ . .	7·0	7·0	10·5	16·3	17·7	32·0	31·6
K_0 . , .	83·3	74·7	97·2	109	72·1	76·7	48 0

It will be seen that, although the p_H is falling slowly, the first order constant which, under similar circumstances in presence of glass wool only, would have been almost constant, with a tendency to diminish, here rises steadily. On the other hand, a zero order constant (K_0) varies little until the hydrogen peroxide concentration has fallen very low. This was found to be typical of all experiments in which coppered glass wool was used.

K_0 = c.c. N/1000 $KMnO_4$ reduced per minute $\times 10^3$.

K_0 in Table 4 is the average value for the peroxide concentration over the range, 5 c.c. H_2O_2 = 12 – 4 c.c. N/100 $KMnO_4$ (see p. 156).

TABLE 4.

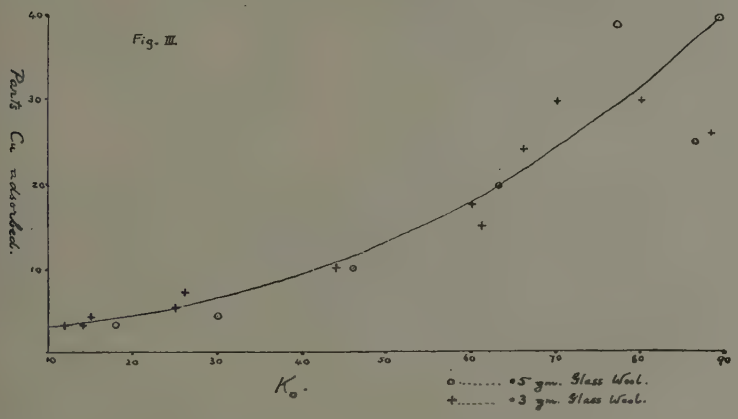
Expt. No.	Glass wool.	Parts Cu in saturating soln.	Parts Cu adsorbed.	K_0.
171	0·3 g.	3	3	14
188	„	3	3	12
176	„	4	4	15
191	„	5	5	25
173	„	7	7	26
162	„	10	10	44
172	„	15	15	61
174	„	18	17·5	60
175	„	25	24	66
164	„	30	26	88
165	„	50	30	70
177	„	40	30	80
187	0·5 g.	3	3	18
178	„	4	4	30
179	„	10	10	46
184	„	20	20	63
180	„	25	25	86
183	„	60	39	77
181	„	40	40	89

When these results are plotted (Fig. 3), it is found that both sets of results fall on the same curve, the value of K_0 depending almost entirely on the amount of adsorbed copper, and very little on the amount of glass wool used. K_0 is roughly proportional to adsorbed copper but does not increase quite as fast.

The change in colour of the copper deposit from green to yellow, which takes place very rapidly when the coppered glass wool is immersed in a solution of hydrogen peroxide, taken in conjunction with the zero order constant, suggests that we are here dealing with a two-stage reaction, a typical case of "intermediate compound" catalysis, the first (very rapid) reaction being the conversion of the adsorbed copper hydroxide into copper peroxide by the hydrogen peroxide solution, the second (slower) reaction, of which the speed is measured, being the decomposition of the copper peroxide. As the concentration of adsorbed copper is constant, the concentration of copper peroxide also remains constant (so long as the hydrogen peroxide concentration is high enough to keep the first stage rapid in comparison with the second), and a zero order constant results.

Several experiments were performed to find whether copper ions in the solution had an effect additional to that of the adsorbed copper. In two typical experiments 0·5 g. glass wool was saturated with adsorbed copper, about 45 parts being taken up. To the peroxide solutions were added 20 and 40 parts of

copper in solution respectively. The zero order constants obtained were 75 and 73, distinctly smaller than the value (about 95) to be expected in the absence of copper ions in solution. In another instance 0·3 g. of glass wool was saturated with adsorbed copper (28 parts), and the peroxide solution contained a further 10 parts of copper sulphate. The zero order constant was 59 (compared with about 76 in the absence of copper ions). The falling off from the theoretical is perhaps not large enough to justify one in classing copper ions as inhibitors, but the figures do suggest that when glass wool and copper sulphate are used together the entire catalysis is of a heterogeneous character. Rudel and Haring (Ind. Eng. Chem., **22**, 1234, 1930) advance the view that copper ions have a marked catalytic effect in acid solutions, but the results quoted in their paper seem to show that, although below p_H 4 an increase in concentration of copper ions increases velocity of decomposition, between p_H 4 and 7 the reverse is true.



In their investigation, moreover, the decomposition of peroxide was followed by measuring the volume of oxygen gas evolved, and it is not stated whether adequate means were taken to prevent supersaturation of the solutions by gas. Results obtained by gasometric analysis in the absence of elaborate precautions to prevent supersaturation must be regarded as under suspicion (cf. Richardson and Bailey, Sci. Proc. Royal Dublin Society, **21** (N.S.), 43, 1934).

The reaction in presence of glass wool bearing adsorbed copper differs in a very important respect from that in presence of glass wool alone. The latter reaction depends to a great extent on the hydroxyl ion concentration of the solution, and increases in velocity as the hydroxyl ion concentration increases. The former reaction is relatively indifferent to the presence of hydroxyl ions, and is, if anything, slower when the p_H is increased.

TABLE 5.

Expt. No.	Wt. of glass wool.	Parts of adsorbed Cu.	C.c. N/100 NaOH.	Avge. pH during 1st hour.	K_0.
From curve . .	0·3 g.	5	nil	7.4	25
167 . .	,,	5	0·75	7·7	23
169 . .	,,	5	4	8·4	21
From curve . .	,,	20	nil	7·3	63
163 . .	,,	20	0·75	7·4	47
170 . .	,,	20	4	9·0	48

SUMMARY.

The investigation confirms the view that hydrogen peroxide decomposes very slowly in the absence of suitable solid surfaces. This was found to be the case even in markedly alkaline solution.

In presence of glass wool, the decomposition is probably complex, and approximately correct results are obtained by using the equation

$$v = 15[H_2O_2]\ [OH']^{\frac{1}{2}} + 7 \times 10^{-5}[H_2O_2],$$

the *amount* of glass wool present having very little influence on the velocity, although the stopping of the reaction in absence of solid surfaces suggests that both first and second order reactions are probably heterogeneous.

Robertson's observation that a filtered extract of glass wool was as effective as the glass wool itself is probably to be explained by the presence in his filtrate of a sufficient number of tiny particles of glass wool to afford a surface for these heterogeneous reactions.

In presence of glass wool on the surface of which copper has been adsorbed the reaction is of zero order, and probably takes place in two stages, a peroxide of copper acting as intermediate compound. Whether such a reaction should be considered as an example of "promoted" catalysis depends on one's definition of promoter action. It seems certain that the combination of glass wool and copper sulphate does not merely enhance existing effects, but that the decomposition on the surface of coppered glass wool follows an entirely different course from that on the surface of glass wool alone, copper ions in solution having little or no effect.

The writer wishes to acknowledge his indebtedness to Miss Isabella H. Stewart, M.SC., by whom a large number of preliminary experiments were performed.

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No. 17.

ON THE PREPARATION AND PROPERTIES OF ALGINIC ACID AND ON THE EXTRACTION OF MARINE ALGAE WITH VARIOUS SOLVENTS (PRELIMINARY NOTE).

By VINCENT BARRY, M.Sc.,

AND

THOMAS DILLON, M.A., D.Sc.,

University College, Galway.

[Read November 27, 1934. Published separately January 11, 1935.]

In a paper published a short time ago from this laboratory (1) the constitution and mode of occurrence of alginic acid were discussed. The alginic acid used in these experiments was obtained from laminariae by the usual methods of extraction of the dried material with sodium carbonate or ammonia. The solutions of alkali alginates obtained by these methods were highly viscous liquids presenting great difficulties in filtration. This high viscosity has been described by all previous investigators of this substance, and has been hitherto regarded as the most characteristic property of alkali alginates. Recent experiments of ours, however, seem to show that it is not a property of any one chemical individual in the plant.

We have been engaged in the extraction of dried and powdered fronds of *Laminaria digitata* with various solvents. The following table shows the solvents in the order of their use, and the approximate quantities extracted by each:—

Solvent.	Percentage of dry fronds extracted.
1. Boiling water	41
2. Methylated spirit	10
3. Methylated spirit containing hydrochloric acid ...	2
4. Ammonia (dilute)	20
5. Boiling dilute caustic soda	20

The first extract was filtered through a glass filter, and gave a cloudy solution of no great viscosity. This solution contained among other bodies the laminarin of Kylin (2). When it was left aside a finely-divided material settled out, which gave a somewhat viscous solution with alkali.

The solid remaining on the glass filter was extracted three times with methylated spirit, and then once with spirit containing a small quantity of hydrochloric acid. When the residual material was extracted with hot dilute ammonia an easily filtrable solution was obtained, which on acidulation gave the characteristic gelatinous precipitate of alginic acid.

At no stage in these operations did we obtain a highly viscous liquid involving the filtration difficulties regarded as characteristic of solutions of alginates.

Schmidt and Vocke (3) and Nelson and Cretcher (4) agree that alginic acid, as obtained by the usual methods (or slight modifications of them), consists of an easily hydrolysable part and a part hydrolysable with difficulty. It may be that the material which comes away in suspension in the aqueous extract corresponds to one of these, while the substance extractable only with ammonia corresponds to the other. This point is under investigation.

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No. 18.

A CRATE FOR THE COLLECTION OF FÆCES AND URINE ADJUSTABLE FOR METABOLISM (SOLID AND LIQUID) EXPERIMENTS WITH PIGS, SHEEP, AND CATTLE OF VARIOUS SIZES.

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Department of Animal Nutrition, University College, Dublin.

[Read November 27, 1934. Published separately January 18, 1935.]

The type of apparatus used for the collection of fæces and urine in metabolism experiments on farm animals is usually adapted for the purpose for one type of beast only. This either restricts experiments to one class of animal, or necessitates the erection of a number of different crates for the accommodation of cattle, sheep, and pigs, respectively. To overcome this difficulty an adjustable construction has been devised and has been in use at the Animal Nutrition Department of University College for the past seven years. With slight alterations it can be made to accommodate pigs and sheep of various ages, as well as cattle ranging in size from a calf to an adult bullock; and so successfully has the apparatus worked that a detailed description of the arrangement appears warranted.

It is clear that experiments of this kind require to be duplicated on similar animals, and consequently a double crate has been provided for.

The compartment in which the crates are built measures 17 feet 9 inches by 13 feet, the door being placed at the centre of one of the longer sides. On either side of the door is a window, a third window being situated on the back wall directly opposite the door.

Figure 1 represents in isometric projection the interior of the compartment, a small-scale ground plan being shown at *A*. Figure 2 is a photograph of the elevated floor of one crate (right) with portion of the elevated platform indicated in figure 1.

The floor of each crate is supported by two iron girders (*HH* in figure 8) running parallel to one another from the back to the front wall. The slips constituting the floor—see figure 1—are laid across the girders. Each slip is 4 feet 9 inches long, and at the end is 3 inches high by 3¾ inches wide. Where it rests on the girders the under side of the slip is flat, but the remainder is shaped as shown in cross-section in figure 1, *B*. As shown in the side view figure 1, *B*, the depth of the slip increases towards the centre, so that fluid falling on it is

diverted towards the centre rather than to the sides. The slips are rounded on top, and are shaved off at the sides, except where they sit on the iron girders, so as to provide a small space between them. All the floor slips except two

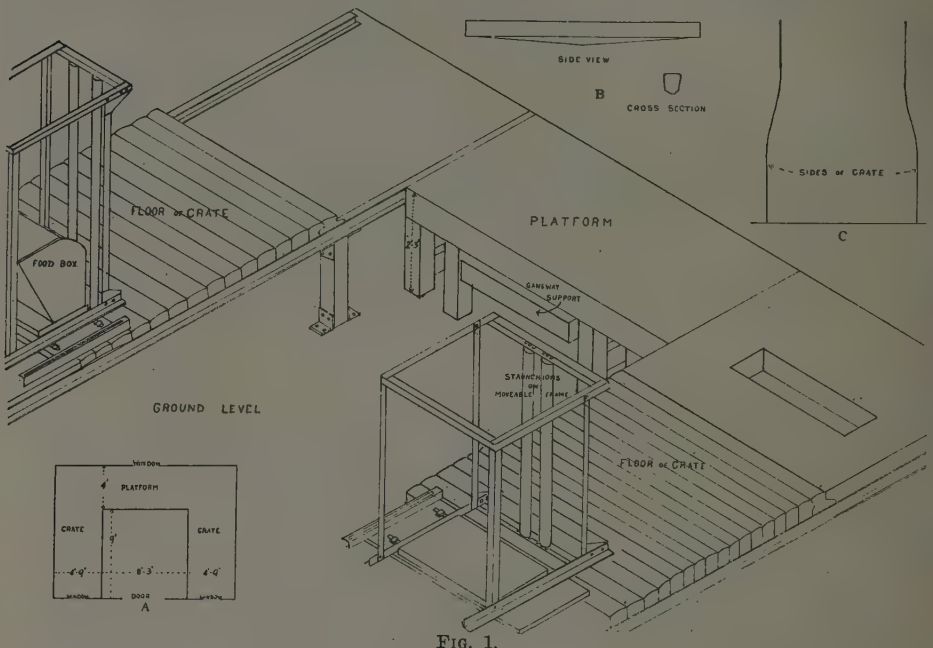

Fig. 1.

are removable. The fixed pair, which are covered with a leaden envelope, are situated 4 feet apart, one being 3 feet 7 inches from the front wall. Twelve removable slips fit in between, and a variable number at either side as required.

Fig. 2.

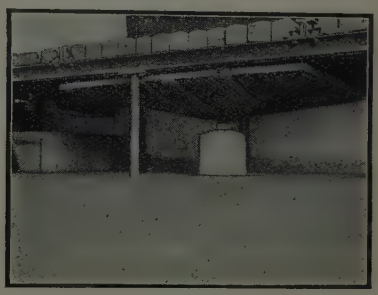

Fig. 3.

Underneath the centre of the floor is a large funnel suspended by slips on the supporting girders on which the funnel is made to slide to the required position.

Figure 3 shows the funnel, which in this particular case is so large that adjustment of position for different sized animals is unnecessary. The emptying pipe at the centre fits into a jar capable of holding 3 gallons. The funnel is lined with lead, as is also the receptacle for the fæces. The fæces trough measures on the inside 2 feet 2 inches by 8 inches at the bottom, and 2 feet 6 inches by 1 foot at the top, and has a vertical height of 9½ inches. There is a handle at one end, as shown in figure 2, which indicates also the position of the trough in relation to the other appliances.

FIG. 4.

FIG. 5.

When an ox is the subject of experiment he is held in position by a pair of cylindrical vertical stanchions, the position of which is adjustable. Figure 4 shows the contrivance which carries the stanchions. It is made of iron, and consists of a fixed platform *P* and a movable superstructure. The latter is

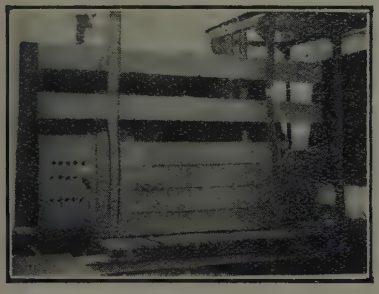

FIG. 6.

FIG. 7.

made to slide backwards and forwards along an extension bar *B* at each side, and also to move right and left along slots at *A* and *A*. In that way the stall may be adjusted in length and in width for oxen of different sizes. The distance between the stanchions can be varied to fit the particular animal

under experiment. The crate for the ox is completed by two sides shaped from the floor upwards as in figure 1, *C*. This, while keeping the standing animal in position, allows him ample room when lying. Figure 5 shows a bullock in position, with the near side of the crate removed, while figure 6 is a picture of the completed crate with the animal inside. The ox crate sides are bolted on top to two parallel horizontal iron beams *M* and *M*, figure 6, which are firmly fixed in the wall, 5 feet 9 inches over the crate floor, 4 feet apart, and which abut over the crate as shown in figure 6. The inner side is fixed, while the outer one can slide on a slot in each wall beam, so that the compartment between may be made to fit the size of the animal. The front vertical bar, *X*, of the movable side fits at the base by a collar arrangement round the front fixed floor slip, *y*, along which it is made to slide into position. It was originally intended to have a similar arrangement for the base of the hinder vertical bar, but this was discarded in favour of a connecting bar, *z*, passing from the inner to the outer side behind the animal's hocks, and pinned so as to prevent the movable side from shifting under pressure from the animal. The feeding is done in a specially constructed movable box shown in position in figures 5 and 6, and pictured with the smaller pig feeding trough again in figure 7. The inner measurement of the base of the bullock feeding trough is 2 feet by 1 foot, and the back slopes outwards. The bottoms of the food and fæces troughs are rounded on the inside. The fæces are directed into the collecting trough by a piece of canvas attached to the animal's body as shown in figure 5. An iron bar in a fold of the loose hanging end of the canvas maintains it taut, and prevents it from getting under the animal's legs. The urine flows freely on to the floor and thence to the collecting jar below. One or more wooden cross floor slips may be placed in position behind the hinder fixed one, so that in this way also the length of the stall may be adjusted to the size required. In the case of figure 5, which shows an eight months old bullock in the crate, one such slip is inserted, but figure 2 shows in addition a special broad slip with a rectangular hole in the centre for the fæces cloth to hang through. This special slip is put into the position indicated so as to provide more rigidity and strength when a large bullock is the subject of the experiment.

For the purpose of getting the bullock into the crate, the movable side having first been placed in position, a removable gangway from the door of the house to the platform at the back allows the animal to walk from the floor to the higher level of the platform and thence into the stall. While the animal is passing into or out of the crate the space at the rear of the floor shown in figure 6 is covered by auxiliary slips as pictured in figure 2. The animals to be used for experiment are first trained to stanchion-tying for some days in one of the farm buildings, and by that means they become quite tractable.

For the accommodation of the pig and sheep a bottomless box which sits on the floor of the crate is provided. The movable side which constitutes part of the bullock crate is removed, the other structures above described remaining

in position as shown in figure 2. Figure 8[1] shows the pig pen in position, *H H* being the iron girders supporting the floor described above. Portion of the side of the pen next the eye is cut away to show the sliding auxiliary head end *B* of the pen, and the feeding trough *A*, which may be placed in or withdrawn from the crate through the doorway in the side—illustrated in figure 10. Both

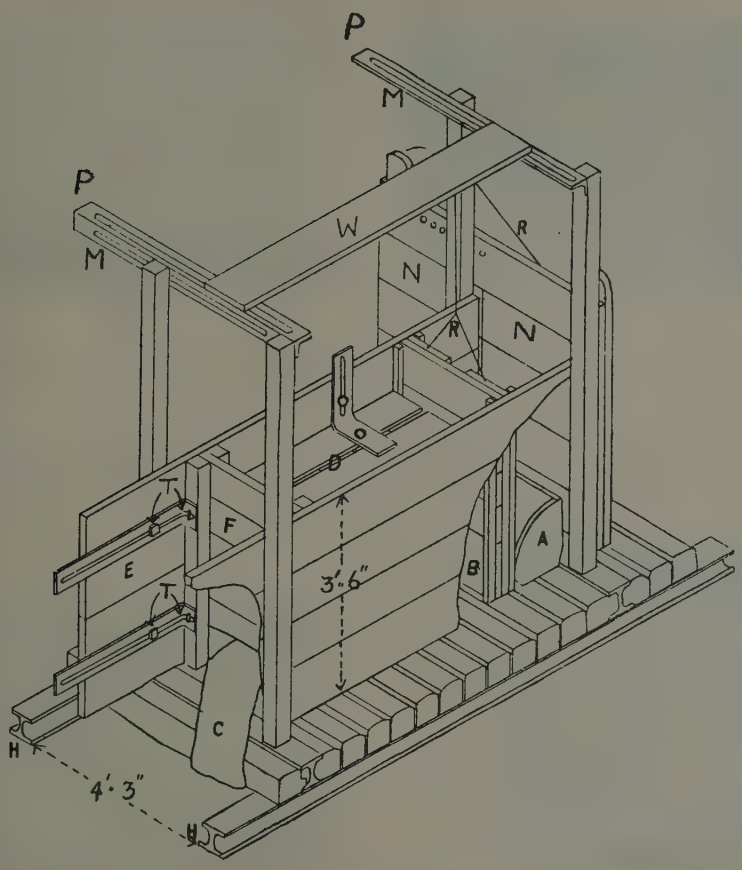

FIG. 8.

sides are bolted to the head end *N*, figure 8, the point of attachment of one side *E* to which is alterable. In that way the width of the pen may be adjusted to the size of the animal. The perpendicular posts which constitute part of the sides are bolted on top to the two horizontal iron beams *M M* fixed in the wall at *P P* and already described in connection with the support of the sides

[1] The drawings in figures 1 and 8 have been kindly prepared by Mr. F. Hussey, B.SC.

of the ox crate. The auxiliary head end marked *B* in figure 8, which is placed 13 inches from the head end proper, *N*, slides vertically between wooden strips, and is manipulated by a rope *R* over a pulley attached to a wooden beam *W*. It is pulled upwards while the pig is eating from the trough *A*, and is dropped back into position when feeding is completed. The left-hand

Fig. 9.

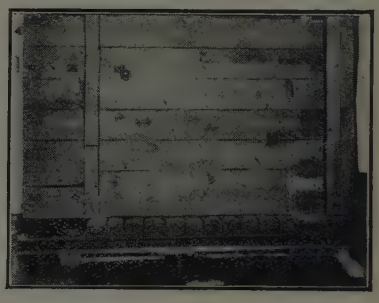

Fig. 10.

object of figure 11 shows the way in which the auxiliary head end is made to extend in concertina-like fashion to fit the varying width of the pen. This is arranged by every alternate horizontal board, a tongue of which fits into a groove in the adjoining boards, being screwed to one vertical supporting strip, the remainder being screwed to the other. To these supporting vertical strips

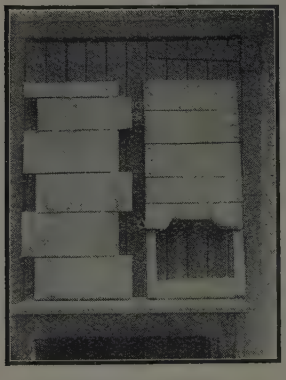

Fig. 11.

Fig. 12.

the cord *R* is attached as seen in figure 8. The rear end *F* of the crate—the right-hand object of figure 11—is held in position by four L-shaped bars (see figure 8), two on each side, which are slotted to receive and slide on the buttons *T T* attached to the sides and rear end of the crate, so that the length of the compartment and the distance between the sides at the rear end may be adjusted as required.

The extent of the accommodation provided to the pig is such as to prevent it turning in the chamber, and also to prevent excessive forward and backward movement. A lid, *D*, adjustable in position and held by buttons in slotted L-shaped bars as shown in figure 8, is necessary to prevent the animal reversing its position. A useful precaution to prevent obstreperous behaviour on the part of the pig is to place a ring in its nose before putting into the crate. As in the case of the bullock the fæces are carried to the trough at the rear end over a canvas cloth attached to the animal's body by a harness as pictured in figure 9. A weight at the distal end maintains the canvas taut, while ropes attached to two ear-like projections at the body end of the canvas—shown in figure 9—and carrying suitable weights over pulleys—pictured in connection with the similar sheep apparatus in figure 12—support the canvas in such a way as to prevent the fæces from dropping over the sides rather than back into the trough.

The pig crate is also used for the sheep, but the auxiliary head end and the lid are dispensed with. Figure 12 shows the sheep in position, one side of the crate having been removed for the purpose of the photograph. As in the case of the pig it gets sufficient room to lie, but not to reverse its position. When the food consists of herbage and water a netted wire rack and water trough are improvised, a sloped boarding under the rack preventing the hay or green material from passing under the animal's legs, while yet not obstructing the sheep in getting into a comfortable position when lying down. A convenient manger could similarly be arranged when meals enter into the dietary.

The slips which form the floor of the crate for all animals were soaked in solignum before being put into use so as to prevent the absorption by them of further fluid. When an experiment is in progress the floor is washed down by a spray of distilled water each day at the time of collection of the urine. The foods used are at the beginning of each test placed in containers on the platform at the rear end of the house, and all weighing and measuring in connection with the food, fæces, and urine are conducted inside the walls of the building.

No. 19.

POLLINATION IN *SAXEGOTHAEA*.

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[Read January 15. Published separately February 21, 1935.]

The commonest type of pollen reception in the Conifers is by means of a pollination drop. A small quantity of fluid, rounded off by surface tension into a spherical drop form, appears at the mouth of the micropyle when the ovule is mature. It is characteristic of more or less exposed ovules in which the micropylar part of the integument is tubular or at least symmetrical. First described by Vaucher in 1841 (13), it is now known in practically all the members of the Cupressineae, Sequoiineae, Callitrineae, Taxineae, and Podocarpineae (Seward's (7) classification). It is usually quite conspicuous on those ovules in which it occurs, and can be seen, if the ovules are not already pollinated, at any period of the day. Few detailed studies have, however, been made of the pollination-drop mechanism since the time of Vaucher, and, although Schumann (6), Fujii (3), and Tison (12), have published interesting observations, we know even now little of many aspects of the phenomenon particularly on the physiological and physico-chemical side.

But the mechanism is not universal. It is now common knowledge from the writings of Thomson (8, 9), Burlingame (1), and Eames (2), for example, that in the Araucarineae the pollen falls on the cone-scale or the ligule, and sends out a tube growing over the scale like a fungus hypha till it reaches the nucellus of the ovule. *Saxegothaea* Lindl. to some degree parallels *Agathis* and *Araucaria*. A monotypic member of the Podocarpineae *S. conspicua* Lindl. forms a definite cone with overlapping scales in a close spiral. The ovules, which early become inverted, are partly sunk in a slight depression of the scale, one such scale being drawn at *a* in the text figure showing the ovule with its epimatium, integument, and the characteristic flaring nucellus protruding through and filling the micropyle.

Pollination in this form has been referred to by Tison (10, 11) and Norén (5). Tison had available two small trees in the Botanic Gardens at Caen, only one of which apparently coned in 1906 and 1908; but, although pollen was freely formed, ovulate cones were very scarce, only four being available in 1906 and "a few" in 1908. He considered that the nucellar flare is definitely

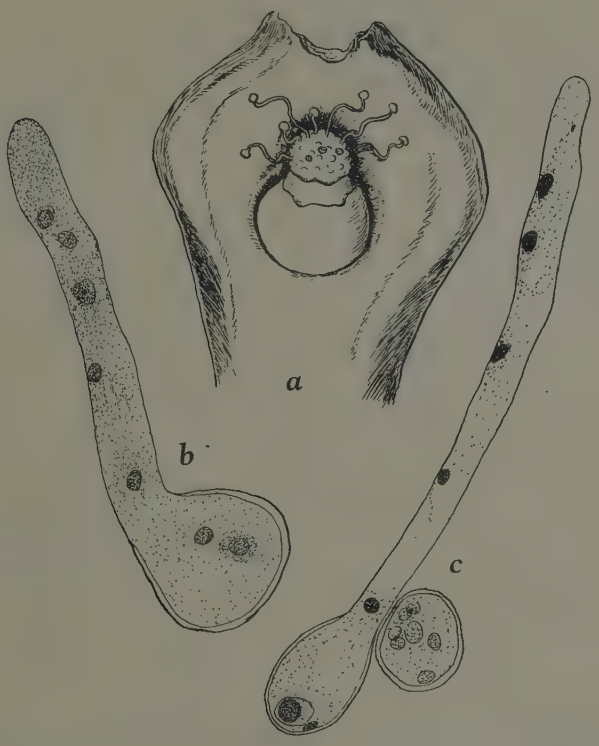
developed as a pollen-receiving stigma, to which the grains adhere due to a slight fluid secretion on its surface — in his own words the flare is "un véritable stigmate nucellaire." He compares it with the nucellar extension of species of *Araucaria* which had at the time only recently been reported, and which he also considered to be stigmatic, although Thomson (8) in 1905 had already recorded the reception of the pollen on the scale of *Araucaria,* with the subsequent development of inch-long tubes. He further claims a certain degree of dichogamy in *Saxegothaea.* "Au moment," he says, "où les étamines commençaient à s'ouvrir les cônes femelles du même pied étaient déjà presque renfermés. Je suppose donc que le *Saxegothaea* est dichogame." He goes on further to suggest that dichogamy may explain the fact that, although thirty ovules were cut in serial section, yet only one single grain on only one flare was seen, even though the ovules were taken from three cones developed on the same branch, "à côté d'un grand nombre de cônes mâles." In this connection our own field observations may be of interest. Two well-growing specimens, about 20 feet in height and coning freely at intervals, which are available at Kilmacurragh, Co. Wicklow, Ireland, do not seem to show this dichogamy in any marked degree. There is perhaps a slight tendency for the female cones on a branch to lag a little behind the male cones on the same branch, but, as the male cones shed pollen fairly actively for about a week, and as there is variation in the maturing of the male cones even in the one clump, and great variation in the maturing of the two sorts of cones on the different parts of the tree, it is clear that, under normal freely coning conditions, when the female cones are ripe, and they certainly remain so for about the 10 days referred to by Tison, there is pollen in plenty in their general neighbourhood. As Tison had only a few female cones available, an exaggerated idea of dichogamy could easily be reached. The absence of pollen grains on the ovules examined by him may be due merely to the accident of the particular cones available being particularly forward, or even to the loss and displacement of the grains in the preparations necessary for the serial section cutting. In the Kilmacurragh trees if sufficient pollen be available all the cones readily secure it from the same tree. There can be no question of pollination from another tree, as the two specimens of *Saxegothaea* are widely separated in the estate.

Norén (5) also considers that the nucellar flare is a functioning stigma, and upon it he found numerous germinating grains. But he goes on to say, "bisweilen konnte ich solche (*i.e.,* pollen grains) auch im Kanal zwischen den Karpiden in einiger Entfernung von der Narbe finden." These grains had sent out long tubes which grew towards the micropyle. No drawing is given by him, nor is any more definite indication given of the frequency of this occurrence than the expression "bisweilen." Tison (11) in his second paper confirms Norén's observations on this point, and records having found "un certain nombre (of grains) envoyant, parfois d'assez loin, leur tube pollinique jusqu'au stigmate," but he obviously still considers this phenomenon of little importance. He figures the only available drawing (in his Pl. IX, 10) of a

germinating grain, but it is one on the flare, with the tip of the pollen tube just penetrating the outer cell layer.

Since Norén, this "bisweilen" phenomenon has been used as one of the arguments to link *Saxegothaea* (and the Podocarps) with the Araucarineae. It is clear, however, from the preceding paragraphs that the basis of this argument is still a little indefinite. Tison had only a few cones available, Norén only one glass-house specimen, the female cones of which he had apparently to pollinate artificially; and both seem to emphasize the essential

FIGURE.



a. Ovuliferous scale of *Saxegothaea* to show, somewhat diagrammatically, the ovule, nucellar flare, and germinating grains; × 20. June 29. *b*. Young pollen tube dissected from the scale showing body-cell in the grain and free nuclei in the tube; × 375. June 25. *c*. Pollen tube germinated in moist air showing the same structure; × 375.

stigmatic function of the nucellar extension. Goebel (4) also expresses the opinion that "bei *Saxegothaea* werden wohl die Mikrosporen unmittelbar auf die . . . Nucellarspitzen gelangen." It seemed desirable, therefore, as plentiful material was available, to decide upon the importance of the phenomenon in

Saxegothaea of pollen reception and tube growth on the scale. It may be said at once that, on our trees growing in the open, the phenomenon is very marked. It is not merely an occasional grain which germinates at a distance from the flare. Actually, within a certain range of influence of it, every grain germinates just where it falls; and ovules may be found with comparatively few grains on the flare, most germinating at a distance, or with few distant grains, or, by far the commonest case, showing the condition outlined in the figure, *a*. This drawing is diagrammatic, but brings out the point that the grains may germinate close to or away from the nucellus growing towards it from all directions, the tubes being long or very short; or the grains may lie on the flare itself. They may even fall on the ovule, growing over it towards the micropyle, and, when pollination is successful, large numbers of tubes, up to 30 or even more, may be found clustered and tangled round the nucellar extension. The ovules sectioned by Tison may thus have had grains near them on the scale but not actually on the nucellus. Norén further thought that fluid must exist between the interseminal spaces to permit pollen tube growth from the distant grains. It is, however, abundantly clear that no such fluid is required, sufficient moisture being available from the water vapour in the practically saturated spaces. This appears both from the dissection of numerous cones at this stage and from the ease with which the pollen germinates in culture. Pollen shaken on a slide which is subsequently kept in a moist atmosphere forms tubes just as long and as rapidly as in nature. Thus *b* in the figure is a drawing of a tube dissected from a scale a short time after germination, while *c* was drawn from a preparation which was obtained by shaking pollen on a slide smeared with egg-albumen, fixing in formalin-acetic-alcohol when suitably grown in a moist chamber, and staining in iron-alum hæmatoxylin. These figures, as well as emphasizing the ease of germination, have another interest. The six nuclei characteristic of the grain of *Saxegothaea* can be seen strung along the tube leaving the body-cell in the grain. Norén mentions, but does not figure, something of this nature. He says that, on growth of the tube, "treten die freien Kerne, alle oder einige, sowie die generative Zelle in denselben über." The generative cell of Norén, it may be remarked, corresponds to the body-cell of other authors, *i.e.*, to the mother-cell of the male-cells. The two drawings here reproduced show that, although the tube and stalk nuclei can hardly at this stage be distinguished from the free prothallial nuclei, the body-cell is quite distinct. It has, however, remained in the tube, this being also the case in nature whether the tubes be short or long. It is hoped to deal more adequately with some of these aspects in a later fuller account of the life-history of *Saxegothaea,* but it is worth further mention here that the development of this plant is spread over two years. Pollination takes place usually in the first week of June, the tubes growing rapidly to the stage shown in the figure at *c*. The tube, with nuclei along its length, and the body-cell in the grain, embeds its tip to a depth of, at most, six cells in the nucellus, and then stops. It over-winters in this condition, renewing its activity in the spring of the following year. It is difficult to follow Norén, therefore,

when he describes, in the tip of the only "older" tube which he found, two nuclei and one cell "die vermutlich die generative Zelle, den Schlauch- und den Stielkern repräsentieren"; especially when he does not indicate what actually is the date of the "older" tube.

It is now suggested that the nucellar modification in *Saxegothaea* is not a stigma primarily. Its primary function is the reception and *retention over the winter* of the tip of the pollen tube which is attracted towards it by some as yet unknown stimulus. This idea of its primary function gives some meaning to the fact that it increases in size after pollination. In fact it only begins to shrivel a little when the pollen tube in the second year has successfully penetrated to the gametophyte. The tube growth and the function of the nucellar tip are thus brought into closer parallelism with the conditions in *Araucaria*, the higher specialisation of the flare in *Saxegothaea* being associated with the long delay in the later pollen-tube development in that form.

Summary.

Pollen grains in *Saxegothaea* germinate wherever they happen to fall, either on, or within a certain radius of influence of, the nucellus flare. The pollen-tubes grow towards it, embed their tips in it a short distance, and then rest in that condition till the ensuing spring. The flare thus has not primarily a stigmatic function but is related to the reception, the retention over the winter, and the belated growth of the pollen-tube tip.

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No. 20.

POLLINATION IN *PINUS*.

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(Plate 3.)

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No complete survey of pollination mechanisms in the Abietineae has been published since Strasburger's (6) account in 1871. There are scattered references in the literature, but in many cases they are incomplete or even inaccurate, while some genera have not been described at all. The recent short survey by Goebel (4) is of value for *Larix* and *Pseudotsuga*, but otherwise advances little on the original observations of Strasburger. This lack of any adequate account of pollination in so important a group as the Abietineae is all the more remarkable since, although the pollination drop of the type so common in the other Conifers is not found, yet there are greater variations within this one group than in the whole of the rest of the Conifer phylum. It is hoped in this and succeeding papers to give some account of these variations. Instead, however, of summarising here the available literature in advance, it is proposed to deal with the genera successively, introducing the pertinent literature in each case as far as necessary.

The structure of the Abietinean cone has to be remembered in considering pollination in any of the genera. It consists, as is well known, of a series of bracts arranged in a close spiral on the cone axis, each bract subtending a scale—the ovuliferous scale—which carries two inverted ovules. Considerable variations in the young cones occur in the relative development and the shape of both of these structures, variations which are on the whole nicely adapted, when the scales of the cone separate a little at maturity, to conduct the pollen to the ovular micropyles. Most of these adaptations have been described in Strasburger's general account, and nothing will be added to this aspect of the problem. The main interest here centres round the subsequent fate of the

pollen, this being the aspect about which the statements are so often inaccurate or confused. The simple point that the ovules are more or less completely inverted, that thus the micropyles may point downwards, is an aspect of importance which has often been overlooked. In general the problem is that of ensuring the *upward* passage of the pollen grain through the micropylar canal. There is no question of the grain *falling down* on the nucellus.

As special interest attaches to *Pinus,* this short paper is confined to that genus, the other genera being dealt with later. The main description deals with *Pinus sylvestris* L., reference being made to other species later on. It is necessary, in the first place, to have a clear picture of the structure of the ovule of the Scots Pine in order to follow the account of the pollination phenomena. Goebel (4, fig. 1909) shows two nice views of the ovule of *Pinus parviflora,* but, although the structure of the ovule of *Pinus sylvestris* is well-known, it is difficult in the recent literature or text-books to find drawings in which its essential form is clearly and simply shown. For this reason two views of the ovule of the common Pine are reproduced in Pl. 3 : 1, 2, the first showing the adaxial, the second the abaxial aspect. The integument is continued as a fairly symmetrical tube well beyond the level of the nucellus. The micropylar opening is relatively large, its diameter being distinctly wider than that of a typical Cupressinean ovule, but the micropylar rim is differentiated. Abaxially the rim usually projects just a little beyond the edge of the scale, running in an even curve, but adaxially it extends further. The edges are produced into two long arms, composed of turgid translucent tissue. There is not much variation in various ovules. The arms may be a little more splayed than in the drawings, or a little curled towards one another, but on the whole the ovular structure is very constant. The rim is also a little turgid and translucent.

As the common Pine has been the subject of intensive laboratory study by students the world over for three-quarters of a century or more, it might be expected that all the details connected with it should be well-known to-day, and indeed most of our introductory text-books usually refer to its pollination. While mention is seldom made in these accounts of any function for the micropylar extensions, the presence of fluid at the micropylar mouth is usually claimed. Thus Coulter and Chamberlain (2) state that "as the pollen is being shed a drop of mucilaginous substance secreted by the upper part of the nucellus appears at the apex of the ovule, and the pollen, caught in this pollination drop, is conveyed to the nucellus." The mechanism of conveyance, however, is not explained. Chamberlain (1), in a recent elementary text-book, reproduces a drawing which actually figures a round drop at the micropylar mouth. As a further example, Skene (5) states that "a drop of mucilaginous fluid is excreted by the nucellus through the micropyle and in this the pollen is caught, the fluid dries up and retracts and the pollen is pulled through the micropyle." Ferguson (3) is one of the few who suggested a function for the micropylar extensions. The pollen is supposed to come into contact with them, they draw together in some way and introduce the pollen into the wide canal. It is not

easy here to visualize the suggested mechanism, which seems to give the arms something of the function of the tentacle of a sea-anemone. Goebel (4) also refers briefly to the arms, suggesting that, though playing no direct part in the transfer of the pollen to the nucellus, they "wohl am Festhalten des Bestäubungstropfens beteiligt sind." Although definite enough at a first glance, all of these and parallel statements are very vague on close examination. Ferguson's suggestion is particularly problematic even without further detailed knowledge of the processes at work, as the arms are clearly intact and unchanged at a time when the grains have already passed through the micropylar canal in an upward direction and have reached the nucellus (see Pl. 3: 4). It was obviously advisable owing to the vagueness of these general accounts to examine the pollination phenomena in greater detail.

It was early obvious that if a pollination drop existed in *Pinus* it must be of a different nature from that of genera such as *Cupressus* and *Taxus*. In these the formation of a drop could hardly be overlooked, but frequent observation of numerous ovules on several trees of *Pinus sylvestris* before and during the pollination period, failed, when the preliminary investigations were being made, to show the presence of any fluid in the ovules. This was reported at the Centenary meeting of the British Association in London in 1931, and the suggestion thrown out that perhaps in *Pinus* no fluid appears, just as no fluid appears in *Larix, Pseudotsuga, Tsuga,* and *Cedrus,* some other mechanism being at work. As the suggestion remained uncontradicted at the time, it seemed likely that few others at any rate had actually seen the fluid. But the problem had still to be solved—How could pollen *pass up* the canal if no fluid were secreted, or further why, if fluid were secreted, its presence was so elusive in the field?

Strasburger (6) first described the secretion of fluid from the ovule of the Pine, and apparently from this paper the later accounts descend. In this article he describes in *Pinus,* in the first place, how neatly the arrangement and shape of the scales facilitate the passage of the grains to the ovular micropyles. This interesting part is, however, seldom referred to in any detail in later references. Having then further described the ovules he refers to the micropylar arms. "Diese dünne Fortsätze werden aus farblosen, glashellen Zellen gebildet die mit Flussigkeit angefüllt erschienen und dieselbe zur Zeit der Bestäubung in grosse Masse secerniren." It will be noticed that he ascribes the secretion of the fluid to the arms, and not to the nucellus, as most of the recent text-books do. This change is probably the influence of work, such as that of Tison (7), on the pollination drop of the Cupressinean type, which seems undoubtedly a nucellar secretion. Continuing, he describes how the pollen grains, guided by the scales, "gelangen, unmittelbar, rechts oder links zwischen die seitlich orientirten beiden Fortsätze der Samenknospe: hier bleiben sie in der secernirten Feuchtigkeit zwischen denselben haften und werden allmählich in das Innere der Samenknospe eingesogen." Such is his full account. No indication is given of the shape of the drop or the area covered by it, or of the duration of the secretion, or of the time taken for the

grains to get inside the ovule, or how they actually ever get in. No other account being found in the older literature it seemed probable that this article of Strasburger was the original source of the text-book descriptions to which reference has already been made. Now it was further just possible that, following, as it did, an examination of the pollination drop in *Taxus,* the turgid appearance of the micropylar arms might have so strongly suggested to Strasburger a secretory function that the secretion was taken for granted although not actually followed out and examined. This is possible, as it is practically certain that the arms have no such active secretory function.

Such was the position when the problem was taken up. As there seems to be no advantage in relating in detail the various steps in its examination, the results can be recorded as shortly as possible. It may be noted, however, that only for about one week in May can field observations be made.

In the first place there *is* definitely a fluid secretion in the Pine ovule at the pollination period, but it is a much more subtle one than in the Cupressinean forms. Even though it may not be found in the field, branches, brought in and kept in water under a bell-jar, may show a copious secretion from most of the ovules of the cone. The secretion may last for perhaps a week, increasing during the first days till a large glistening mass is formed, mainly situated on the higher edge of the rim, filling the arms, invading the lower side, obviously filling the micropylar canal, and even joining with that from the nearest ovule on the scale beside it to form a mass of water sufficiently heavy to overcome the surface tension and run down the cone axis. The secretion of the fluid, however, gradually stops and the whole eventually dries. In the field conditions are very different. The young cones bursting from the bud scales seem immature physiologically. Although apparently well formed, with good ovules and fully developed arms, they do not secrete fluid till kept under a bell-jar for about two days, while cones which have been freed from the bud-scales for about that period, if unpollinated, readily secrete fluid if brought indoors to a saturated atmosphere. The young cones are thus exposed for a period. In *Pinus sylvestris,* if the trees are coning well, both pollen cones and ovulate cones will be found at all stages—some fully mature, others obviously young. Thus, except in the case of the very few ovulate cones which come out first and earlier than the others, there is always a considerable quantity of pollen flying, especially in a clump of trees, when most of the ovulate cones get freed from the bud.

During the immature period, therefore, when the young ovules cannot be induced to secrete fluid, they are still being plentifully dusted with pollen. This pollen adheres readily to the projecting arms, sometimes in sufficient quantity almost to cover them if the pollen shed is very dense. The arms are unquestionably of a stigmatic pollen-holding nature. Although mainly to be found on the arms, some grains may also be found adhering to the rim. Unfortunately it was not possible to secure a satisfactory photograph to illustrate this point from nature. In the meantime the ovules have matured to the stage when the secretion of fluid can begin. This secretion, at least in the material

of *Pinus sylvestris* available to us, seems to be a night phenomenon. Hundreds of cones have been examined at all day periods during three springs, but in only two ovules was any fluid seen, and then only a slight trace of moisture deep in the micropylar canal. On the other hand, if cones were examined at night, fluid can be seen with the greatest of ease in practically every mature unpollinated ovule. The night hours have not been followed as closely as the day hours, so it cannot be stated how soon after night-fall the secretion begins, or how long it remains during the morning hours, but at about 2 a.m. a very typical secretion is abundant and at 7.30 a.m. every ovule is dry. The fluid is probably secreted by the nucellus. It appears first deep in the canal, gradually filling this to the level of the rim. A small quantity then becomes drawn out by surface tension into a film stretching to about one-half the length of the arms. It is very unusual to find it reaching to the tips. Goebel's (4) suggestion that the arms may help to support a pollination drop is thus in general correct, although it is clearly inadequate so to describe the secreted fluid. The film itself or the fluid about the rim thus naturally comes into contact with at least a few grains, two or three will suffice. The grains are readily wettable, and become at once detached from the arms or rim, and enclosed within the surface of the fluid. Examination, however, of the distribution of the grains about the ovule suggests very strongly that, in the vast majority of cases, it is in the film that the pollen becomes enclosed. The stigmatic arms to which the grains adhere thus induce also the film development by which the grains are eventually seized. While not quite certain it seems probable that the entry of the grains in many cases causes a slight but rapid increase of surface tension, resulting in the withdrawal of the film, with the enclosed grains, back nearly to the level of the micropylar rim again. The winged pollen is very buoyant, floating easily and rising quickly in water. As the ovules are inverted, and the canal filled with fluid, the grains naturally begin to rise, thus traversing the micropylar canal in an upward direction.

The markedly erect position of the young cones is probably in relation to this. Irrespective of the lie of the branch from which they arise, the early spring growth from the bud is always strongly positively geotropic, giving the Pines in spring a very characteristic appearance. The young growth may subsequently come to lie horizontally, or may even droop, but at first it is always erect, surmounted by the young ovulate cones which are rigidly erect. In this the Pines differ from many Cupressinean forms, for example, in which the young female cones may take up varying positions, in most forms tending to a strong inversion. The erect position of the young cones is characteristic of most of the Abietineae, *Tsuga* and *Picea orientalis* being interesting exceptions. In the case of the Pine, if grains are introduced into the fluid of an ovule, the cones being kept horizontal or oblique, the rising grains can frequently be seen to jam against the micropylar rim, and may even be left stranded in that position. Owing to the capillary dimensions of the canal the rise is relatively slow but quite definite. This mechanism alone ensures their reaching the nucellus, but this object is furthered in a quite spectacular manner.

As the grains are rising, probably in most cases before they reach the nucellus, although there is a little doubt on this point, the fluid begins to lessen in amount and to be reabsorbed. The time taken for this reabsorption naturally varies a little, but the average time for complete reabsorption, so that the grains now rest on a dry nucellus, is something less than ten minutes. Absorption usually definitely sets in within a minute and a half; within five minutes the fluid has well disappeared into the canal, complete drying occupying another couple of minutes. On occasion absorption is more rapid. Disappearance of the fluid deep into the canal has been noticed in two or even in one minute, complete drying taking only five minutes or less. This phenomenon of reabsorption has been examined very frequently both in ovules exuding under a bell-jar in the laboratory and in the field. It is interesting in the field at night artificially to introduce a few grains into the fluid of one of the ovules of a scale, leaving the other ovule of the same scale unpollinated, the difference after a five minute interval being striking, as the unpollinated ovule retains its fluid unchanged. The mechanism is in general very effective. If normal quantities of pollen are available it is difficult to secure unpollinated cones for indoor examination unless carefully selected from the very young cones just out of the bud and still immature. All the ovules of practically every other cone will be found to be pollinated, so that, in all probability, if pollen has reached the arms, one exudation of fluid in one night ensures effective pollination, and, if once the fluid has been reabsorbed under the pollen stimulus, renewed secretion definitely never takes place. The cellular physiology underlying the phenomena presents a pretty problem as yet unfaced.

It should be understood, however, how much in this account is definite observation and how much is conclusion from experimental pollinations. The secretion of fluid particularly at night; its distribution at the micropylar mouth; the rapid reabsorption of the fluid induced by the introduction artificially of a few grains into it; the delay in the maturing of the ovules in the field permitting the deposition of pollen on the arms before the first secretion of fluid; the dusting of the arms with pollen in the laboratory resulting in the later appearance of grains on the nucellus as in the field—these points are quite definite. The actual stages in the secretion of the fluid and its gradual filling of the canal at night have not actually been followed step by step in the field, although ovules can be readily found with varying amounts of fluid secreted, especially during the early night hours. The film formation between the arms is obviously a rapid surface tension phenomenon, and if pollen is present on the arms, its subsequent retraction is rapid also. The act of rapid seizure of the grains, in Nature, by the film or by the main fluid mass is thus the only point which has not been seen. But can any other conclusion be drawn from these observations?

In addition to this general account, a few points require further consideration. If an ovule secretes fluid when no grains have yet been caught, or if the grains are lodged on some part not accessible to the fluid, it is possible that, if pollen be then flying, it may be caught in the fluid itself. From a

consideration, however, of the relative numbers of ovules with pollen-dusted micropyles before secretion, and of unpollinated ovules on trees with pollen flying in plenty in which the secreted fluid can be still seen at night, it is fairly certain that in *Pinus sylvestris*, at any rate, the catching of pollen directly by the fluid is an occurrence of comparatively little importance. A further question also arises in connection with ovules which mature early before pollen is flying, or which mature on trees with little pollen. These show a typical exudation at night, and are again dry during the day. It is probable that the night secretion can be repeated in the field for about three nights in succession, but our observations on this point are not complete enough for a decision, as, unfortunately, it is only for a few days that field observations can be made in any year. It is hoped to settle this point next spring. But clearly there is no necessary rhythmic reabsorption and secretion, as cones kept in a moist atmosphere retain the secretion on the ovules over the whole of the 24-hour period, and actually show an increase in the amount of the secretion during the first few days, related probably to the low evaporation. It is of interest here that these larger diluted masses of fluid react much more slowly to the introduction of pollen grains; and if many days old may show very little reabsorption or none at all. The fastest reabsorption is shown in the field by young ovules in which the fluid is just sufficient to be flush with the micropylar rim, and to send the film up between the arms. The day and night change in nature may, therefore, be associated with changes in the evaporating power of the air, and corresponding changes in the water-content of the whole plant tissue, but the elucidation of such points as these is for the future.

The winged grains also need a further comment. No satisfactory function has yet been attributed, as far as we know, to the wings on the pollen grain of *Pinus*. They can hardly be an advantage, as is commonly suggested, to facilitate spread of the pollen, as the very much larger—at least twenty times larger—grains of *Larix* and *Pseudotsuga* are wingless, and yet are disseminated just as effectively as those of the Pine. The pollination mechanism just described gives one clear function to the wings, they float the grain in water. All wingless grains sink readily. The grains floating with horizontally distended wings rise in the micropylar canal, and are kept also from too intimate contact with the sides by just this horizontal extension of the wings. It is suggested that the wings may have a further value. Not only do they cause the grains to float, but they cause them to float in a definite way—with the wings up. The prothallial cells are thus on the under side of the grain, the side away from the nucellus as the grains rise in the canal. The pollen tube later bursts out from that part of the grain away from the prothallial cells, thus from that part which, owing to the manner of its flotation, tends to be brought to rest in contact with the nucellus. The tube on germination could thus penetrate the nucellar tissue immediately and directly. If the grains are numerous they probably jostle one another in the final settling and may be somewhat displaced from the position, so convenient for germination, outlined above.

An ovule, with part of the integument removed, is drawn in Pl. 3: 3. It shows the condition about ten minutes after the introduction of pollen into the film, with the grains now lodged in the well-known shallow depression at the top of the nucellus. Pollination thus successfully effected is followed by closure of the micropyle. The cells a little below the rim grow mainly on two sides, this swelling closing the micropyle inwardly, at first to a slit and later to complete obliteration of any opening. Ferguson (3) and Strasburger (6) among others have dealt with this closure, but there is still much obscurity in connection with the detailed cellular activity. The same lack of detail is to be noted in the other members of the Abietineae that close in a similar way, but this question is out of the scope of this account. The external bulging appearance at this stage is shown in Pl. 3: 4, which also shows that the arms have played no very active part in the pollination. They are still intact but beginning to fade. Their function was solely a passive one.

Observations have been made on several other species of *Pinus*. Some little variation is seen in ovular structure, but none depart very markedly from the condition as seen in *Pinus sylvestris*. The micropyle, as seen laterally, of *Pinus tabulaeformis* var. *yunnanensis* Dallim, is shown in Pl. 3: 5, as an example of one of the more elaborate types, but it is clearly of the same nature as in the common Pine. The pollination mechanism would seem also to be essentially the same in the various species except in one particular to which attention might be drawn. It would appear, at any rate under cultivation, that dichogamy may be a regular feature of some forms, although it can hardly be said to be so in *Pinus sylvestris*. Ovulate and pollen cones on the same branch undoubtedly may not mature simultaneously in this species, but there is such a variation, spread over about a week, in the maturing of the various cones on one tree that, even with trees growing in masses, most of the ovules can be pollinated from the same tree at least in calm weather. Only the few earliest ovulate cones, which mature before any pollen is shed, would ordinarily be pollinated from another tree; and still fewer ovulate cones, if any, seem to mature after the pollen has all been shed. Under forest conditions it is probably only in strong wind that cross-pollination takes place to any extent. The conditions are very different in other species, for example, *Pinus Mugo*, Turra and *Pinus tabulaeformis* Carr. The former species is protogynous, the latter protandrous. *P. Mugo* at a time when none of its own pollen is flying, or only a very little on really rough shaking of the branches, commonly shows the ovulate cones mature for pollination, well freed from the bud, scales fully separated, and every detail of structure clearly visible. The maturity of the ovules is also shown by the readiness with which fluid is secreted when brought into saturated air under a bell-jar. In this species, also, the day retraction of the drop in the field is not quite so complete as in *Pinus sylvestris*. In the morning hours up to 9 a.m. it is always possible to find occasional ovules in which the typical secretion can be seen just flush with the micropylar rim, or even still with the film between the arms; but such ovules are still sufficiently rare to need an active search, and, as the day advances, it becomes increasingly

difficult to find one. It was the discovery of these which originally led to the clearing up of the phenomena in *Pinus sylvestris*. Owing to the longer duration of the fluid in *Pinus Mugo* it is possible also that pollination may be more often effected by grains caught directly in the fluid, the stigmatic function of the micropylar arms and rim being of less importance than in the common Pine, although the frequency with which pollen can be found on the arms of ovules which have no grains on the nucellus shows that the stigmatic function still plays a considerable rôle in the pollination. Observations in its native habitat or in cultivation under forestry conditions are necessary before it can be said that cross-pollination is characteristic of this species, but such is strongly suggested. It is of interest that pollen of *Pinus sylvestris* can be used to induce the rapid absorption of the fluid in *Pinus Mugo*. The reciprocal crossing is equally effective. The protandry in the other species mentioned, *Pinus tabulaeformis*, is so complete that it is felt it must be due to conditions of cultivation. When the pollen is flying in clouds the ovulate cones are still small in the bud, and the first tinge of red bursting through the scales only appears when the pollen is completely shed, the staminate cones being withered and brown for some days before the first of the ovulate cones can be considered mature. The night secretion could easily be found in many ovules at least ten days after it was judged that the pollen had been completely shed. Fluid exudation can be found in the morning hours in an occasional ovule of this species, but this phenomenon, although a little more frequent than in *Pinus sylvestris*, is decidedly less so than in *Pinus Mugo*.

It would seem desirable to extend such observations and to determine fully the rôle of dichogamy in Conifer pollination. We have available a number of records but mostly incomplete; and any record to have value must obviously be related to natural or sylvicultural conditions. But if we knew the facilities for hybridization in the field we might be in a better position to decide on the true nature of the suggested hybrid swarms in *Abies* and *Picea*.

Summary.

1. The pollen grains of *Pinus sylvestris* adhere to the micropylar extensions which are essentially stigmatic.

2. A fluid secretion gradually fills the micropylar canal to the level of the rim and runs up as a film between the arms.

3. This secretion is a night phenomenon and, in unpollinated ovules, can seldom be found during the day; but may be secreted for several nights in succession.

4. Pollen, already on the arms, is caught in the fluid which is then quickly reabsorbed, within ten minutes or less.

5. The wings of the grain are shown to have an important function in relation to the pollination mechanism.

6. Marked dichogamy is shown by other species of *Pinus*.

We wish to express our thanks to J. W. Besant, Esq., Keeper, Botanic Gardens, Glasnevin, Dublin, for free access to the valuable collection under his care.

EXPLANATION OF PLATE 3.

[All drawings by Miss M. O'Leary. Magnifications are approximate.]

FIG. 1. *Pinus sylvestris* L.—Adaxial view of complete ovule with part of scale and bract. × 80, May 28.

FIG. 2. *Pinus sylvestris* L.—Abaxial view of micropylar part of the same ovule. × 140, May 28.

FIG. 3. *Pinus sylvestris* L.—Ovule dissected to show pollen grains in the nucellar depression. × 30, June 9.

FIG. 4. *Pinus sylvestris* L.—External abaxial view of ovule to show the swelling due to the growth of the closing cells, and the arms still present but withering. × 30, June 19.

FIG. 5. *Pinus tabulaeformis* var. *yunnanensis* Dallim.—Micropyle of ovule as seen from the side. × 140, May 25.

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FIG. 1.

FIG. 2.

FIG. 3.

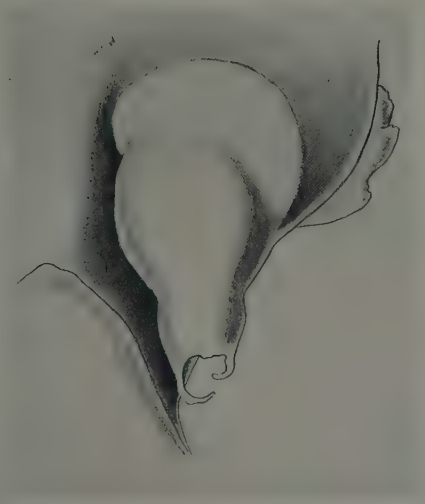

FIG. 4.

FIG. 5.

No. 21.

POLLINATION IN *TSUGA, CEDRUS, PSEUDOTSUGA,* AND *LARIX.*

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(Plates 4 and 5.)

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This paper continues the survey of pollination phenomena in the Abietineae which was begun in the preceding paper, in which *Pinus* (4) alone was described. The four genera now dealt with show three markedly different mechanisms quite distinct from the *Pinus* type, but all agree in one particular—the absence of any fluid exudation from the ovule associated with the securing of the pollen; and for that reason are here dealt with together. Among the other genera *Keteleeria* is not available, and, as some aspects are still uncertain in *Picea, Abies,* and *Pseudolarix,* it is hoped to deal with these later when the data required have been obtained in the spring of 1935.

The structure of the Abietinean cone in relation to pollination was referred to when dealing with *Pinus* (4), particularly the simple fact that the ovules are inverted on the ovuliferous scales in relation to the morphology of the cone axis, the micropyles thus pointing more or less downwards if the cone is erect.

Tsuga.

The only reference available to pollination in this genus is in the paper by Strasburger (13) on pollination in the Conifers already frequently referred to in the account of *Pinus.* It occurs in a foot-note to his account of *Picea,* which, he says, *Pinus canadensis* resembles. This is presumably *Pinus canadensis* Bong., an old name for *Tsuga heterophylla* Sarg. From the context it is not quite clear if he refers to ovular structure and the manner in which the pollen reaches the nucellus, or merely to the general adaptations of scales and bracts; presumably the latter, because the ovular structures of *Picea* and *Tsuga* are very different. Two species of *Tsuga* were available in good coning condition, *T. heterophylla* Sarg. and *T. Brunoniana* Carr., in both of which the pollination phenomena are essentially similar. The pollen is shed some time in the first fortnight of May, according to the species, and shows the first peculiarity of the genus in bearing no wings, sinking readily in water like all

other non-winged grains. The young female cones also differ from most other Abietinean cones at pollination in one particular. These latter, coming out from the bud, either stand markedly erect on the branch which bears them, or are kept in the erect position by a marked positively geotropic growth of the young branch which carries them, as in *Pinus*. In *Tsuga* the cones are carried terminally on rather lax branchlets, and lie partly horizontally or actually drooping but at no particular angle. The ovules, therefore, although inverted in relation to the cone axis morphologically, actually lie, spatially, either horizontal or with the micropyle pointing somewhat upwards. The indefinite lie of the ovules of *Tsuga* is of importance, not so much in itself, as in emphasizing the spatially inverted ovules of a form like *Pinus*. The ovule is a simple one among the Abietineae. The micropyle is short and more or less symmetrical with neatly scalloped edges (Pl. 4: 1). At the time of pollination the apex of the nucellus, which is rounded, lies a little below the micropylar edge, as is seen in the dissected ovule of Pl. 4: 2, but, in the course of about two weeks, it increases in size a little and becomes pointed, with the apex practically flush with the micropyle. It never seems actually to protrude, nor does its tissue seem more specialized to form a receptive area than the nucellar apex of many other Conifers.

The point, however, of greatest interest and importance is that the pollen never reaches the micropyle directly. Some grains reach the neighbourhood of the ovules, but most lodge on the upper surface of the scales or the lower surface of the bracts. The pollen then germinates *in situ*, the tubes growing towards the cone axis whatever the position of the cone. It is not yet quite clear why so many roundish unwinged grains lodge on the scales; there may be a slight mucilaginous secretion, but the point was not determined with any certainty. Frequently a larger number of the germinating grains are to be found on the abaxial or under surface of the bracts than on the scales. After pollination, when the cone closes, the scales are pressed against those bracts next above, and—whether it be due to lesser development of cuticle on the bract surface is still uncertain—many of the grains bore into the bract to a slight depth, and grow tangentially to the surface for a short distance. They then commonly emerge again, but continue their growth more closely pressed to the bract surface, and appear mainly on it when the cones are dissected. The point is, however, not general, and is of minor importance. No endeavour is made to bring it out in the drawing in Pl. 4: 3, in which an impression is given of the appearance, looking in towards the cone axis, when some of the scales have been removed. The drawing is diagrammatic to this extent. Only a very small number of the tubes which may be found in a well-pollinated cone have been included, as it proved impossible to incorporate them without obscuring the general appearance; and those drawn have been widened for the sake of clearness. Naturally among many of the scales of many cones comparatively few grains have been lodged, and the tubes are correspondingly few; but, if three to ten times the number of much finer tubes be considered as present, the drawing can give some impression of the appearance seen in a well-pollinated

cone. The tubes can be found growing in masses, sometimes in straight parallel bands, sometimes twisting and intertwined, sometimes branching, although that is rare, till they reach the neighbourhood of the ovules. Here some of the tubes growing on one bract or scale may advance directly to the nearest ovule, and, slipping over the micropylar edge, penetrate the nucellar apex; but just as commonly, others may grow across the intervening spaces to other ovules, or overshoot an ovule and then turn back. This occupies from one and a half to two and a half weeks after pollination. In the succeeding weeks the micropyle very slightly increases in length, and thickens a little, pressing in the pointed top of the now withering nucellus, but the opening is not completely closed till the whole structure later begins its proper development into the seed. It is remarkable that Murril (9) overlooked this interesting phenomenon when working over the life-history of *Tsuga*.

Many points clearly call for further examination beyond the mere observation of the facts. In the gamut of the reproductive history of any higher plant, there are a series of cellular phenomena which we only know from dissection or the microtome, but of which, from the fundamental biological point of view, we know nothing; the morphological interest has swamped the physiological importance of the sequence of events. Apart, however, from these deeper questions of morphogenesis, an obvious problem here is to determine, for example, why the pollen of *Tsuga* germinates so readily on the scale, while that of *Pinus* will not. It frequently germinates even within the pollen sac, a rather unusual condition in the Conifers, while proving more difficult to grow in culture than the corresponding pollen of *Saxegothaea*. Why, too, do the tubes slip so nicely over the micropylar edge to penetrate the nucellus? But such problems are, for the present, outside the scope of this series of papers. There is one point of observation, however, which is still uncertain. Probably only a limited number of pollen-tubes enter any one ovule, and many, presumably those which have grown more slowly, thus reaching satisfied ovules only, seem to grow on more or less indefinitely for some time. These, or some of them, may penetrate the cone axis, or other tissues, as in *Agathis*, but this has not yet been satisfactorily determined.

It is not proposed to enter here into any speculation concerning the possible phylogenetic origin and development of the pollen-tube in the Conifers. But considerable emphasis has been laid on the similarity between the phenomena of pollen-tube growth on the scale in *Saxegothaea* and the Araucarineae. Although the suggestion of a protosiphonogamic pollen-tube growth in *Saxegothaea* was originally based on rather slender evidence, it has been shown, in a recent (3) short paper on the pollination in that genus, that the contention is well-founded. Within a certain radius of influence of the nucellus the pollen germinates where it happens to lie, the tube growing over the scale towards the ovule; and a drawing is given to show the free prothallial and other nuclei passing down the tube, as happens in the Araucarineae. As the nucellar flare is probably not really a stigmatic or pollen-catching device but developed for the reception and retention of the pollen-tube, the resemblance, whether

indicative of relationship or not, between the pollen-tube phenomena in *Saxegothaea* and the Araucarineae is shown to be even closer than could be considered from the original evidence. A very obvious interest is thus associated with this discovery of protosiphonogamy in an Abietinean genus. The tubes, of course, in *Tsuga* are of varying length. Some pollen lodges well down near the cone axis, and the tubes from these grains are naturally short, but, in the case of the grains further out, the tubes, although not quite so long as those of *Araucaria,* and less spectacular than those of *Agathis,* are still markedly longer than those of *Saxegothaea*; and in this connection one further comment seems necessary. It has been claimed, by Eames (5) for example, that the supernumerary nuclei in the pollen-grain and tube of the Araucarineae, and presumably also in those of *Saxegothaea,* are not of any phylogenetic importance but are associated with the peculiar powers of hypha-like tube growth in these forms. In *Tsuga,* however, only the nuclei typical of any Abietinean pollen-grain and tube are to be found, thus rendering Eames' criticism less acceptable. The fact, also, of such scale growth of pollen-tubes in four genera of the Conifers cannot be ignored in present considerations of the origin of the stigma and of the general carpel condition and pollen-tube phenomena among the primitive Angiosperms.

Finally, it would appear that the wingless pollen-grains of *Tsuga* and the indefinite position of the cones point unmistakably to the importance of the wings in *Pinus* and the erectness of its cone, and lend strong support to the suggestions put forward, in the account of this latter genus (4), concerning the part played by these two factors in pollination.

Cedrus.

Strasburger (13) refers indirectly to *Cedrus Libani,* quoting Parlatore, who says that it resembles *Picea.* This probably refers, as in *Tsuga,* to the general cone structure. Smith (12), in dealing with the development of *Cedrus,* refers to the ovular structure, and comments on certain difficulties related to pollen growth. His account of the young micropylar structure, derived apparently from paraffin sections, is inadequate, as will later appear; the other difficulties are readily explained when the phenomena associated with pollination and subsequent development are examined. Saxton (11) has published a short note on the pollination of *Cedrus* with some drawings of the young ovules. His description of the ovular structure is, however, rather short. He speaks of a one-lipped integument, which apparently is of a stigmatic nature, the pollen adhering to the concave surface of the lip, which is somewhat helmet-shaped. This is not quite clear, perhaps even a little misleading, although the drawings, especially his Figs. 1 and 2, bring out the structure quite well. The interest of Saxton's note, however, lies in the claim that the ovule of *Cedrus* has a micropyle with a stigmatic function. Later stages were not examined by him, but he permits himself to say that "it is not unlikely that the grains get detached from the integument in nature at a later stage and fall into the base of the

micropyle." As the ovules are more or less inverted there is, of course, the obvious difficulty that the grains under these conditions would have to fall *up*; it is certain that there is some further mechanism to be followed out.

In examining the developments at, and subsequent to, pollination there was difficulty in locating, in the neighbourhood of Dublin, trees of any of the three forms of *Cedrus* which could be relied on to yield supplies of ovulate cones at accessible levels. We wish to thank H. G. Carter, Esq., Director of the Botanic Gardens, Cambridge, and F. G. Preston, Esq., Superintendent of the Gardens, for supplies of cones of both *Cedrus atlantica* Manetti and *C. Libani* Loud., supplies which enormously supplemented our own more meagre material. Without this assistance we could never have become satisfied on the conditions in *Cedrus*. The drawings and description which follow refer to *Cedrus Libani*, the Atlas Mountains form being very similar in all respects.

A comment is necessary in the first place on the pollen of *Cedrus*. It is a little remarkable that it is not possible from a reading of the recent literature to decide without personal examination on so obvious a feature as the presence or absence of wings on the grains. Goebel (7) and Dallimore and Jackson (1) state definitely that the pollen is wingless, while Pilger (10) and Beissner-Fitschen (6) say that wings are present. Saxton (11) combines both in his note on *Cedrus* pollination by speaking, in his description, of wings on the pollen, but drawing the grains without wings in his illustration. It is, therefore, well to put on record again that wings are definitely present; it is clear that their omission from Saxton's drawing is merely a slip of the pen. But there is one further peculiarity about the grains. Attention was called when dealing with *Pinus* to the buoyancy of the grains. When stirred in water, for example, they all rise very rapidly to the surface. The grains of the Deodar likewise float, but the grains of the Atlas Cedar show considerable variations. Some float, some sink, and some remain suspended for a considerable length of time; and it is easy to see, in many of the grains that sink, that the wings are somewhat more poorly developed than they are in the Pine. As the sequel will show, it is difficult to attribute any rôle in pollination to wings on the grains of the Cedar, and it is suggested that they are vestigial structures already showing signs of disappearance in the genus. The position and lie of the ovulate cones also seem to show a relation to the pollination. In *Pinus* these cones are rigidly erect when leaving the bud. In *Cedrus* they are usually described as erect, and, undoubtedly, examination of groups of cones on the branches establishes the general accuracy of the statement, but, if the young cones be considered more carefully, it appears that the vertical position follows, not from any marked geotropic tendency, but from their position on the branches. The main lateral branches are, on the whole, horizontal, and the various dwarf shoots tend to stand more or less vertically and at right angles to the branch. As the young ovulate cones continue accurately the line of direction of the dwarf shoot which carries them, most of them naturally assume a practically vertical position. On downwardly sloping branches, however, the cones will commonly be found in an oblique position relative to gravity, although

still at right angles to the main branch. As these cones mature they seem later to show a slight geotropic response, but the final position seems really determined by the lie of the dwarf shoots from which they arose. There is in the Botanic Gardens here a strongly drooping variety of *Cedrus atlantica* in which most of the branches hang vertically downwards, the dwarf shoots lying frequently in a horizontal position. The young ovulate cones lie therefore very obliquely, sometimes nearly horizontally, even the older mature ones seldom making an angle with the ground much greater than 60°. It is probable then, and the sequel seems to confirm it, that the erect position of the cone of *Cedrus* is related to the habit of the trees, and is not an adaptation to pollination as it is in *Pinus*. The general erect position of the cone, however, as it determines an inverted position spatially of the ovule, clearly puts out of consideration any possibility of the pollen falling down the micropyle.

The ovule, as it appears about the pollination period, is shown adaxially and abaxially in Pl. 4: 4, 5, which, with the drawings in Saxton's paper, bring out the structure at this stage. The micropyle bends back and outwards a little over the edge of the scale, and becomes splayed out into a relatively wide notched flange which shows considerable variation in detail. The flange is always well developed adaxially, but the notching about the outer side of the ovule is often so deep that the flange appears as prolonged into two broad blunt processes; on the other hand the whole edge may be only slightly scalloped. The figures show a common intermediate type. Abaxially the flange is usually more entire, and, although still well splayed, not quite so wide as on the adaxial side, and curved over more steeply; but, again, the appearance may vary, and ovules are frequent in which the abaxial side is prolonged into a blunt process, giving a very two-lipped appearance to the micropyle. Smith (12) says, "one side of the integument is much longer than the other and is folded inwards to form a groove." We cannot relate this last expression to anything seen by us in ovules of this date, and the relative sizes of the two sides of the integument as seen in section will clearly depend on the plane of the section and the degree of variation in the particular ovule cut. Saxton (11) speaks also of "the concave side of the helmet-like integument" and illustrates this with a figure. This description and drawing rather overemphasizes the appearance, especially about the time of pollination. The micropyle as a whole certainly turns back over the edge of the scale, but this is mainly done by the neck, the flange, splayed out all round, lying in a more or less vertical or very oblique plane. Saxton's Fig. 4 does not suggest this rather even splay of the micropylar edge. The inner side of the micropyle, however, always narrows into a rather long slit running a considerable distance along the edge of the ovule. But, again, in any ovules examined by us we have never seen the broad gap at the side of the ovule as drawn in Saxton's Fig. 3. The slit seems rather to contract very rapidly, further narrowing as it is followed down the ovular edge, till it is difficult frequently to decide where it definitely stops; nor, even when the scale is dissected away, do we find it, except very rarely, running sufficiently far down the ovule to expose the nucellus, at this stage very small. Saxton's

drawing, however, brings out the relatively small size of the nucellus in the ovule about the pollination period.

There is no question but that this splayed-out micropyle is stigmatic. It is composed of much more succulent and translucent cells than, say, the micropylar neck. It is still uncertain if it secretes any fluid; if such occurs it can only be a very fine film indeed, and it is just as probable that the cell-walls are sufficiently mucilaginous to cause the pollen-grains to adhere; but these points have not yet been examined. The pollen-grains do adhere, however, in numbers, as shown in Saxton's drawings and in Pl. 4: 5.

The subsequent phenomena are of interest. Saxton thought it possible that the grains become detached later, and fall into the base of the micropyle. This, of course, they cannot do. Smith found, shortly after pollination, numerous grains between the scales but rarely one within the micropyle, a condition paralleled apparently up to May of the next year. He was clearly unaware of the stigmatic nature of the micropyle. Saxton also describes in his own material that the preparation for embedding and cutting washed the grains off the micropyle, some few of which were caught in the micropylar neck. Probably this happened also in Smith's material, thus accounting for his non-discovery of the grains on the stigmatic flange, and the occasional occurrence of non-germinating grains within the micropyle. Actually what occurs is this. The ovule remains practically unchanged throughout the winter with the grains till adhering to the splayed flange. As the ovule is inverted if the grains fall from the micropyle they will fall out of the ovule. In May a rapid increase in size of the ovule takes place, particularly in its main body and in the nucellus, the micropylar area increasing only slightly. The abaxial part of the latter indeed contracts a little, displaying now rather a rounded edge; even if originally drawn out into a blunt tongue-like process this turns back and contracts, remaining as a poorly discernible appendage to the rounded edge. Again, owing to the great variations in the detailed shape of the young flange, the subsequent appearance varies, but, on the whole, the adaxial part of the flap, which remains prominent or even increases a little, tends to curl over on the abaxial edge. A fairly common appearance of the micropyle area at this stage is outlined in the drawings, Pl. 4: 6, 7, 8. The first two of these show the abaxial view. Part of the scale has been dissected away in each case, and the curled-over adaxial part of the micropyle has been lifted up a little. The third drawing is of the outer aspect of the ovule a little towards the adaxial side, and shows the appearance of the curled over flap from this angle. But, as stated above, considerable departure in detail from these shapes are met with. The pollen-grains are still held scattered over the inner surface of this turned in part (Pl. 4: 6). The drawings also show how greatly the basal part of the integument has grown, the slit so prominent in the young ovule now appearing in many cases as little more than a notch. They show, too, how the nucellus has also grown, its tip being plainly visible where it lies on a level with the slit. Frequently, owing presumably to the pressure of the surrounding growing areas, it seems somewhat flattened, and even tends to project laterally through the

slit a little. The appearance of the nucellus at this stage is shown in Pl. 5: 9, the integument having been partly dissected away. It narrows rapidly towards the top, and is continued then a short distance to end in a rounded tip. These four drawings are all from ovules of May 25th. As the nucellus presses down further towards the micropylar mouth its upper portion becomes modified as shown in the dissected ovule drawn in Pl. 5: 10. The narrowing, where the main mass passes into the upper cupola-like part, is perhaps a little less marked in the fresh condition, as this was drawn from a fixed ovule. The extreme tip contracts to a slightly swollen apex with large swollen and turgid cells. This tip comes into immediate contact with the pollen-grains which are still adhering to the turned over micropylar flap, or at least approaches them very closely. Whatever the stimulus may be, the pollen-grains touching or nearest to the tip now send out their tubes. The drawing was made from a collection of June 18th, when the tubes had already penetrated the nucellus. The tubes having successfully entered deeply into the nucellus, the upper part of this contracts and, as shown in the dissected ovule drawn in Pl. 5: 11, seems drawn out to a thin extension. This extension is referred to by Smith (12) and drawn in his Fig. 5. He described it as "extending far up into the micropyle whose opening is lateral to the long axis of the ovule and turned towards the ovuliferous scale." It is drawn as if it were an obliquely inclined, more or less symmetrical, micropyle, but the actual appearance will clearly depend on the lie of the section. As Saxton has pointed out, it is very difficult, unless, of course, proper reconstruction from serial sections is resorted to, accurately to visualise ovular structure from microtome slides alone; but, from the account of the development already given, it is easy to appreciate the relations in the section drawn by Smith. If we consider the ovule of May 25th, drawn on Pl. 4: 8, which shows the bending over of the adaxial part of the micropyle as seen from the outer side, and, if it be imagined a little more developed as it would be in July when the nucellus shows its greatest elongation, it is clear that, if cut in the plane of the drawing, that is practically at right angles to the scale, it would show the appearance of Smith's Fig. 5, but would depart from it if cut in other planes.

Although not yet subjected to experiment it seems that this mechanism in *Cedrus* is independent of the cone position, and gives no function to the wings of the pollen-grains. If the wings of pollen-grains are really, as suggested by *Pinus*, adapted to flotation, not in air, but in fluid, the suggestion that they are vestigial in *Cedrus* may be temporarily accepted; and this genus shows a general parallelism with *Tsuga*, in which also no fluid exudation from the ovule took place, and which is characterized by completely wingless pollen, and a very indefinite lie of the young ovulate cones.

Pinus, *Tsuga*, and *Cedrus* thus show three very different mechanisms; but *Pinus* and *Cedrus* have this in common that the micropylar part of the integument has in both a definite stigmatic function. This pollen reception by a stigmatic micropyle shows, in the Abietineae, its most elaborate development in the genera *Larix* and *Pseudotsuga*. In these, one edge of the micropyle becomes

largely swollen, forming an almost spherical protuberance composed of turgid translucent cells, frequently with projecting papillae. On this stigmatic area, very definitely recalling the stigma of many an Angiosperm, the pollen is caught. The area collapses and contracts, then, turning in all round, introduces the pollen into the top of the micropyle. These facts are now well known, the two genera having recently been fairly fully described by Goebel (7) and Doyle (2), among others, and only a few further comments on each will be made here.

PSEUDOTSUGA.

The common Douglas Fir, *Pseudotsuga taxifolia* Britt., being available in quantity, is the only species of the genus which has been examined. In common with the other species recorded it possesses large wingless grains, and further tends to show a less erect position of the ovulate cones at pollination than is found in *Pinus*. Many of the cones can only be described as sub-erect, and those at the extreme ends of some of the branches may be almost horizontal. The remarks made in connection with wing function and cone position in *Cedrus* and *Tsuga* apply here also.

As no drawings of the extraordinary ovule of the Douglas Fir seem readily available three views are reproduced in Pl. 5: 12, 13, 14. The adaxial view is shown in 12, a more or less abaxial view of the micropyle area with the scale partly removed in 13, and a lateral view of the inner side in 14, again with the scale partly dissected away. In the two latter views the swollen stigmatic area has been raised up to show the much smaller abaxial lip upon which, in nature, the slightly overlapping large stigmatic mass is pressed down, the line of contact being a hardly discernible slit. These drawings supplement the descriptions already given, but again variation may be found. The appearance shown in Figs. 13 and 14, in which the two lips are continuous on the inner side but separated by a slit on the outer side, is the common type and the one already described, but this outer interruption may not be in evidence, the two sides being rather similar; and various intermediate forms may be met with. The collapse and incurling of the large stigmatic protuberance after pollination have also already been described, but further observation shows a slight omission in that description. As the stigmatic area collapses the whole micropyle elongates a little, the edge turning in all round so that it soon appears almost symmetrical. This is shown in section in Pl. 5: 15. The condition is exactly paralleled in *Larix*, there being so little difference between the two genera that the drawing of *Larix*, at the corresponding stage, in Pl. 5: 18, shows quite well the form of the unsectioned micropyle area in the Douglas also. It is well known that the pollen remains at the top of the micropyle, more or less entangled by the hair-like outgrowths to be seen on the inside of the inturned rim and which have resulted from the collapse of the stigmatic expansion. Some of the grains, however, are to be found later a short distance down the micropyle, but none get at all near the nucellus. Lawson (8) seems to suggest that they are prevented from reaching the nucellus by the contraction and

kink which appear some distance down the micropyle neck, and which reduce the canal to a slit-like passage. This, however, can hardly be the case. In the first place, the narrowing of the micropyle is not as marked as suggested by Lawson, a space quite wide enough for the passage of several pollen-grains being left. In the second place, the ovules in many of the cones are actually inverted spatially at pollination, so that only during the subsequent inversion of the cone could the grains fall down the micropyle. Even if the cones all lay horizontally the grains in some of the ovules could only fall a short distance down the micropyle as a result of the oblique lie of the upper part of it; and the subsequent complete inversion of the whole cone, which brings the actual ovules into an erect position, is so slow, and the grains so small, that, moist as they are, it is clearly unlikely that they can slip any distance during the process. During the closure, however, some do become detached from the inturning rim and slip a little along the micropylar canal. The inverted position of so many ovules combined with the angle, but not the narrowness of the micropylar canal, puts a limit to their further passage. The grains, as is also well-known, ultimately germinate *in situ* sending their pollen-tubes through the lumen of the micropyle fairly directly to the nucellus. As the pollen-grains of the Douglas Fir are among the largest in the Coniferae, tubes, dissected out at this stage, form excellent material for the examination and demonstration of early growth of the Abietinean pollen-tube, the body-cell being very massive and distinct.

There is, however, a considerable delay in the germination of the grain. The young cones, erect or sub-erect during pollen reception, remain so for perhaps a week, and then slowly begin to bend downwards, becoming markedly inverted, the process being completed in about three weeks from the original pollination. As far as can be ascertained no tube growth begins until a few days after inversion is complete. By this time the whole micropyle tube has increased considerably, especially in thickness, the kink referred to has become largely obliterated, and, except in the case of grains tucked very completely under the remnants of the inturned rim, the tubes can grow directly downwards, as the ovules now, owing to cone inversion, stand spatially erect. It is tempting, of course, to suggest that some gravity stimulus is here effective, but as no experimental work has yet been carried out any suggestion is out of place. It is common also to find grains which have lodged on the scale, usually those resting in the groove between the ovule and the scale, germinating as they lie; and others which had been lodged so far back on the stigmatic expansion that they were not enclosed by its subsequent incurling, may frequently be found in early growth stages. These grains also seem to show no tube growth until cone inversion is complete; but they never develop for any length of time and die in about seven to ten days. During the growth of the functioning tubes within the micropylar canal the cells of the tissue are very succulent and turgid, and there is possibly an exudation of fluid within the micropyle, but the whole question of the growth of the grains, both in the ovule and *in vitro*, has still to be investigated.

Larix.

This genus so closely resembles *Pseudotsuga* in its large wingless pollen-grains and its general ovular structure that only a short further comment is necessary. The ovular structure has frequently been described in general, earlier references being given in Goebel (7) and Doyle (2). As, however, no good drawings are available in the recent literature two views are here reproduced; and, since variations occur in the ovular structure of some of the species, which do not seem yet to have been fully recorded elsewhere, the drawings are those of *Larix Kaempferi* Sarg. It is hoped to deal with the variations in the other species separately. The ovule of the Japanese Larch is, however, very similar to that of *L. decidua* Mill and *L.* × *eurolepsis* A. Henry.

The adaxial and abaxial appearances are shown in Pl. 5: 16, 17. The stigmatic expansion, while still large, is not quite so massive as in *Pseudotsuga,* nor is the abaxial lip developed as in that genus, this side of the micropylar rim being comparatively smooth and level for more than half the circumference. In addition to the account of the closure already described (2) it may be noted that the whole micropylar rim takes part, a growth and incurling taking place all round as in *Pseudotsuga,* so that very shortly after pollination the exterior of the micropylar mouth shows the symmetrical appearance presented in Pl. 5: 18. The kink, accompanied by an internal contraction of the canal, as described for *Pseudotsuga* (8), is indicated also, but the contraction is less marked and more ephemeral than in that genus. But *Larix* differs markedly from its ally the Douglas in one important point. The cones are erect at pollination, perhaps a little more rigidly so than in the Douglas Fir, but unlike that form they remain erect till maturity. The ovules thus remain inverted, but the only germinating pollen found, or recorded from other accounts, appears on the nucellus. Pl. 5: 19 is reproduced for comparison with the corresponding stage of *Pseudotsuga* in Pl. 5: 15. The grains clearly have to pass the micropylar canal in an upward direction, although they are large and wingless and sink readily in water. Unfortunately we have not yet been able to decide how that is brought about. The following mechanism is tentatively suggested pending further observation. Fluid frequently may be found apparently filling the micropylar canal. This fluid may loosen the grains and, then retracting, bring these with it, held by the strength of the surface film; but the mechanism does not seem a very satisfactory one. It is astonishingly common, even if grains are on the nucellus, to find other grains left behind at the top of the micropyle, and, in many cases, two months or more after pollination numerous swollen but ungerminated grains may be found still adhering to the unturned rim but none on the nucellus. No slightest protrusion has ever been noticed coming from these, contact with the nucellus is clearly necessary for pollen-tube growth. This has been found both in the Japanese and the European Larch, but how far it may depend on seasonal influence and perhaps differ from year to year or place to place is not known; certainly the tree of *Larix Kaempferi* in the Botanical Gardens here has only

a very small percentage of ovules in which any pollen ever reaches the nucellus, and very seldom have more than two grains been found germinating on one nucellus, in spite of the fact that the stigmatic swelling of the micropyle may be literally covered with pollen in March. *Larix* thus introduces still another problem in Conifer pollination to be solved in the future.

Summary.

1. Ovular structure in relation to pollination is examined in *Tsuga, Cedrus, Pseudotsuga,* and *Larix.*

2. No pollination drop or fluid is exuded in any of these.

3. In *Tsuga* the pollen lodges on the bracts and scales, the tubes growing toward the micropyle of the ovule as in *Araucaria.*

4. In the other three the micropyle has a very definite stigmatic function.

5. In *Cedrus* the pollen is caught on a flaring micropyle which bends over a little, the nucellus growing down to come into contact with the grains there held.

6. In *Larix* and *Pseudotsuga* the pollen is caught, as is well known, on a large swollen stigmatic development on one side of the micropylar edge. Further observations are added to those already published on these genera.

7. Some of the general problems arising are briefly discussed.

In addition to those mentioned in the text, sincere thanks are returned to J. W. Besant, Esq., Keeper, Botanic Gardens, Glasnevin, Dublin, for access to the valuable Conifer collection under his care, and to the owners and gardeners of the estates of Kilmacurragh and Powerscourt, Co. Wicklow, Ireland.

EXPLANATION OF PLATES.

(All drawings made by Miss M. O'Leary. Where ovules are inverted they are so drawn. Magnifications approximate.)

Plate 4.

Fig. 1. *Tsuga Brunoniana* Carr.—Ovule showing simple micropyle with a slightly flaring scalloped edge. × 20, June 16.

Fig. 2. *Tsuga Brunoniana* Carr.—Ovule with integument partly removed to show the bluntly pointed nucellar apex almost at the level of the micropylar opening. × 20, June 16.

Fig. 3. *Tsuga Brunoniana* Carr.—Small area of dissected cone showing pollen-grains on the scales and bracts, with tubes growing over these to the ovules. × 20, June 16.

FIG. 4. *Cedrus Libani* Loud.—Ovule, shortly after pollination, viewed adaxially showing the micropylar extension. × 60, November 10.

FIG. 5. *Cedrus Libani* Loud.—Stigmatic micropylar extension of ovule viewed abaxially with pollen-grains adhering to it. × 60, November 10.

FIG. 6. *Cedrus Libani* Loud.—Apical region of older ovule showing the turned over adaxial part of the micropyle slightly raised to display the pollen. × 45, May 25.

FIG. 7. *Cedrus Libani* Loud.—Similar view of another ovule. × 45, May 25.

FIG. 8. *Cedrus Libani* Loud.—Lateral view of another ovule at the same stage. × 45, May 25.

PLATE 5.

FIG. 9. *Cedrus Libani* Loud.—Ovule at the same stage as Figs. 6, 7, 8 dissected to show shape of nucellar apex. × 20, May 25.

FIG. 10. *Cedrus Libani* Loud.—Still older ovule dissected to show nucellar apex when contact is made with the pollen. × 20, June 18.

FIG. 11. *Cedrus Libani* Loud.—Ovule dissected to show the long pointed nucellar apex some time after the pollen-tubes have well grown. × 10, July 18.

FIG. 12. *Pseudotsuga taxifolia* Britt.—Adaxial view of ovule at pollination stage showing large stigmatic swelling of the micropyle. × 60, April 2.

FIG. 13. *Pseudotsuga taxifolia* Britt.—Abaxial view of stigmatic micropylar area, the large swelling raised to show the opening and the second smaller lip. × 80, April 2.

FIG. 14. *Pseudotsuga taxifolia* Britt.—Lateral view of ovule to show more fully the relative size of the adaxial stigmatic swelling. × 80, April 2.

FIG. 15. *Pseudotsuga taxifolia* Britt.—Diagrammatic view of older ovule dissected showing symmetrical closure of the micropyle and pollen-grains forming tubes both inside and outside. × 15, June 22.

FIG. 16. *Larix Kaempferi* Sarg.—Adaxial view of ovule. × 40, March 23.

FIG. 17. *Larix Kaempferi* Sarg.—Side view of ovule. × 40, March 23.

FIG. 18. *Larix Kaempferi* Sarg.—Complete ovule shortly after pollination showing symmetrical closure. × 25, April 25.

FIG. 19. *Larix Kaempferi* Sarg.—Ovule dissected to show grains in a nucellar depression. × 15, June 11.

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FIG. 1.

FIG. 2.

FIG. 3.

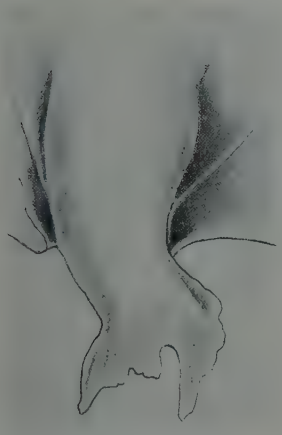
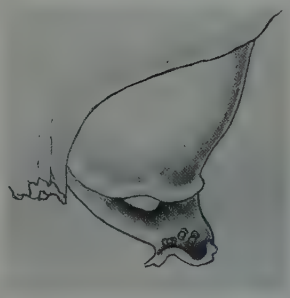


FIG. 4.

FIG. 5.

FIG. 6.



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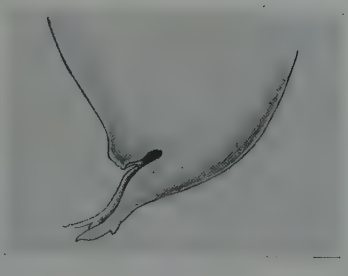
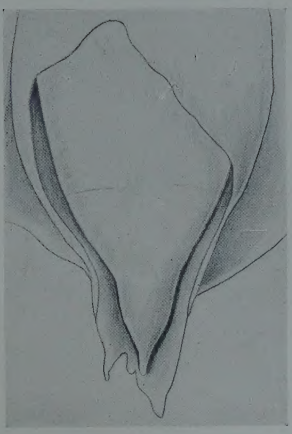


FIG. 8.

FIG. 9.

FIG. 10.



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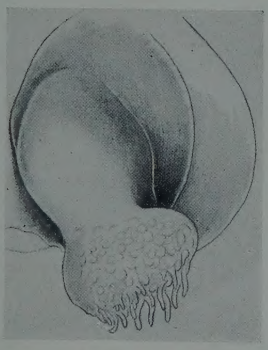

FIG. 12.

FIG. 13.

FIG. 14.

FIG. 15.

FIG. 16.

FIG. 17.

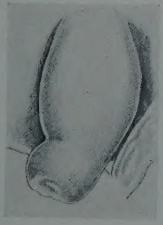

FIG. 18.

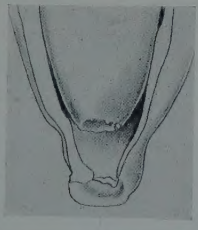

FIG. 19.

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